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Dr. H. Tietz:

The ^{32}P Marked Diethyl Thionophosphoric Ester of β-Oxyethyl Thioethylether (Active Ingredient of the Systemic Insecticide "Systox"), Its Absorption and Translocation in Plants 1

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BAYER LEVERKUSEN (GERMANY)
Department for Plant Protection

The ^{32}P Marked Diethyl Thionophosphoric Ester of β-Oxyethyl Thioethylether (Active Ingredient of the Systemic Insecticide "Systox"), Its Absorption and Translocation in Plants

By Dr. H. Tietz

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A. Introduction

In 1948 the diethyl thionophosphoric ester of β-oxyethylthioethylether, bearing the internal factory designation "E 1059", was synthesized by Schrader (1952). Very soon afterwards this compound was found to possess extremely favourable properties showing it to be a highly effective systemic insecticide (cf Unterstenhöfer 1950: „Über den gegenwärtigen Stand der inneren Therapie" ["The present level of inner therapy"]). In the Bayer research laboratories, the preparation which contains 50 % active ingredient "E 1059" and 50 % "emulsifier 233" (polyethylene glycol ether), was given the number "8169". It was subjected to thorough tests both in the laboratory and outdoors. Plants watered with aqueous solutions of "8169" absorb the active ingredient through the roots and translocate it to the organs above the ground. Within 2 to 6 hours after the application of the preparation, the whole shoot is strongly toxic to parasites with piercing-sucking mouth extremities. In the greenhouse the toxicity of the plants lasts anything up to four weeks. When the preparation is sprayed on plant parts growing above ground similar results may be obtained although the toxic effect is restricted to 10 to 14 days. Approximately 5 to 6 weeks following the application, the treated plants and fruits are no longer toxic to warm-blooded animals. When applied in the usual concentration of 0·01 to 0·1 % preparation content in aqueous solution, no damage is inflicted upon the plants. Other large-scale field tests were carried out between 1949 and 1951 not only by the Bayer research institutes but also by public institutes. All these tests ended successfully. The preparation "8169" was put on the market in Spring 1952 under the designation "Systox". This meant that plant protection at last had a product available which largely meets the requirements for an "inner therapy of the plant".

The possibilities offered by this preparation with its insecticidal efficacy and the particular value it has as a prophylactic against virus infections in plants have been described in numerous test reports and publications. It would be difficult to discuss these at this stage. (Bosse 1952; Dosse 1952; Hofferbert and Orth 1952; Unterstenhöfer 1951, 1952; Zattler 1951.)

B. Experience so far gained with synthetic, systemic insecticides, in particular those with radioactive tracers

Prior to the publication of papers dealing with investigations on systemic insecticides, a precise report on the behaviour of the insecticide "E 605" had already been issued by Frohberger in 1949. He established in his studies that the preparation is absorbed by the roots but by no means in the same quantities applied to the roots. "E 605" is only slightly soluble in water (approx. 1 : 20 000), and is electrically indifferent. For these reasons, according to Frohberger, it is not able to pass through the roots like nutrient salt ions, although in view of the lipide-solubility of the preparation the roots cannot hinder its penetration. Which path it takes to do this had to be left undecided. Whether the active ingredient only uses the cell membranes to move in the plant or whether it also penetrates to the cytoplasm and the cell sap is a question as yet unclarified. Another factor not yet proved is whether the active ingredient can be inactivated in the cytoplasm of the root. One point, however, has been established, namely that the preparation can be traced in the guttation fluid. Those quantities which reach the green leaf tissue via the transpiration stream are inactivated fermentatively in the tissue. All observations point to the fact that the propagation of "E 605" is a matter of pure diffusion processes. It also penetrates green leaves. The question of transportation in untreated parts of the plants can be eliminated simply because diffusion processes do not take place over long distances, and also because the active ingredient, even though it is translocated on reaching the conducting strands, very soon yields to an inactivation in the green leaf tissue. The investigations of Lüdicke (1949) on leaf miners proved beyond all doubt that the preparation possesses a penetrative property. Here also the diffusion is very slight so that the insecticidally effective substance only spreads on a small scale.

In 1951, David and Gardiner published a detailed paper on studies they had made on the systemic effects of four preparations, namely

1. "Schradan" = octamethyl-tetra-pyrophosphoric acid amide
2. Fluorophosphoric acid-di-dimethyl amide
3. "Mintacol" ("E 600") = O,O-diethyl phosphoric acid-O-p-nitrophenol ester
4. Sodium fluoroacetate (salt of monofluoroacetic acid).

The effect of these four preparations is determined in the biological test against aphids. Following an application to the roots the compounds show the following increase in their toxicity in the plants to sucking insects: $4 > 2 > 1 > 3$. It is dubious whether "E 600" is absorbed by the intact roots. The effective period of "Schradan" and sodium fluoroacetate in the plant is considerably longer than that of the other two compounds. The most unsatisfactory chemotherapeutic index is that of the fluoroacetate. On applying the substances to the leaves of

Vicia faba plants, a strong systemic effect may be observed with the Na-fluoro-acetate. This may only be achieved with "Schradan" provided drops are constantly applied to the leaves for several days. The eradication of aphids on the untreated parts does not commence until the 7th day onwards, and only to a very small extent. Fluorophosphoric acid-di-dimethyl amide and "E 600" reveal no translocation effects whatsoever. With respect to "E 600" the authors hold the insolubility in water as being responsible for the absence of a systemic action.

In the same year (1951) D a v i d examined the same substance, containing radioactive phosphorus, on the plants, and arrived at the following result. The radioactive "Schradan" is absorbed by the roots of a *Vicia faba* plant, placed in a culture solution, only as long as "Schradan" is still present in the solution. However, the concentration of "Schradan" rises constantly in the offered solution. From this it is assumed that a significant resistance is offered to the absorption of "Schradan" through the roots. The plants show the greatest radioactivity in the roots which means that although the active ingredient is distributed throughout the whole of the plant, the roots retain the highest concentration value. Plants absorb the substance at a much slower rate from garden soil than from sand. The introduction of the insecticide by means of the cut taproot technique shows that approximately 50 % of the radioactive "Schradan" is broken down inside the plant within a period of 8 days. The concentration of "Schradan" not broken down must be approximately 50 mg/kg beans in order to completely eradicate *Aphis fabae*. Killed aphids were found to contain approximately 15 to 20 mg/kg radioactive material. The honeydew of the aphids is likewise radioactive. Absorption and translocation of the radioactive material takes place when applied to the leaves of *Vicia faba*, cabbage, hops, peas and gooseberries. In the case of *Vicia faba*, the radioactive material can be traced in the interior of the leaf provided it is applied to the upper surface of the leaf a few hours beforehand. On all the plants investigated, it was found that the radioactive substance can also be traced in the untreated parts. The translocal effect is greater in the younger plant parts, although it is also present in the older parts. In favourable cases, when either a large number of leaves had been treated or a large quantity of active ingredient had been applied, it was found that the insecticidally effective quantities moved to the young, untreated parts of the plants. Wherever the plant had absorbed the active ingredient through the roots, no measurable quantities of radioactive material were transpired.

In 1952, D a v i d published a paper on studies he had made with radioactive fluorophosphoric acid-di-dimethylamide on the plants, whereby ^{32}P was also selected as a radioactive atom. The author endeavoured to draw comparions with the previous tests. The radioactive substance is absorbed by the roots of *Vicia faba* from culture solutions. The activity in the plant increases as the solution is absorbed, and in the washed roots it is greater than in the rest of the plant. The radioactivity of the remaining solution, calculated on the volume, shows a relatively greater decrease. The plant, therefore, had shown a preference to absorb the substance from the solution. In this respect, this substance differs fundamentally from "Schradan". The material is absorbed at a much slower rate by plants growing in soil than by those growing in sand. An estimable portion of the active ingredient, absorbed by the roots, is again exudated in the form of vapour by the leaves. The transpired material can be collected. It is radioactive and insecticidally effective. Aphids which suck on the treated plants become radioactive.

Fluorophosphoric acid-di-dimethyl amide is not so lipidesoluble as "Schradan", and does not penetrate particularly well into the leaf of *Vicia faba*. Since it is exudated from the plant during the process of transpiration, only slight quantities are translocated in *Vicia faba*, hops and cabbage following an application to the leaf.

C. Formulation of the problem

From the above expositions, it is to be assumed that substances with insecticidal properties, which are translocated in the plant, form groups of substances more or less related to each other. Apart from gradual differences in toxic efficacy, these compounds do, however, differ greatly in their chemical and physical properties. The water and lipidesolubility differs in each case. To what extent the absorbability is dependent upon this cannot be determined so readily. Most of these substances do not produce any specific bioassay reactions. This applies particularly to the bioassay in the plant. The presence of such a preparation can only be determined by biological test methods, although in this case only approximate quantitative assertions can be made. If the chemical is subjected to autolytic processes, or, because of the plant's own substances, to inactivation processes, then the results cannot be accepted as reliable. Fission products can only be investigated by means of the biological test provided they themselves show a toxicity of a more or less equal degree.

If, on the other hand, the molecule is marked with an artificial, radioactive atom, such as was the case in the studies carried out by D a v i d (1951 and 1952), the presence of the preparation can be determined with a greater degree of exactitude. The only question left to be clarified is whether the measured quantity of substance is actually present in its original chemical constitution.

In view of the fact that all the preparations so far examined have a specific behaviour, it would appear necessary also to examine the active ingredient "E 1059", which has so far produced the best results as a systemic insecticide, with a radioactive, marked molecule. In doing so, it would be of interest to clarify the following problems:

1. The penetrativity into the plant.
2. How does the translocation in the plant take place?
3. Are there any storage organs for "E 1059" in the plant which might explain the long period of effectiveness?
4. Which factors govern the exudation of the preparation? In answering this question it should be established, if possible, what the extent of exudation is when it commences, and what degree it reaches.
5. To what extent does the plant store residues which may be of special significance with respect to the later utilization of the plants as food and fodder for humans and animals respectively?

In all the investigations it should not be forgotten that the preparation, with respect to its behaviour in the plant, represents a substance which is not specific to the plant, and which should not be compared with nutrient salts or assimilates that are capable of movement.

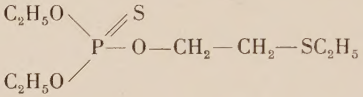
D. Materials and methods

I. The active ingredient "E 1059"

a) Chemical, physical and toxicological data

(All data are taken from the monograph by G. Schrader, 1952, "The development of new insecticides on the basis of organic fluorine and phosphorus compounds".)

The active ingredient, bearing the internal factory designation "E 1059" and the trade-name "Systox", represents chemically the diethyl thionophosphoric ester of β-oxyethyl thioethylether, and has the following structural formula:



Boiling point: 2·2 mm/121° C

Density: $d_{4^0}^{21^0} = 1{\cdot}119$

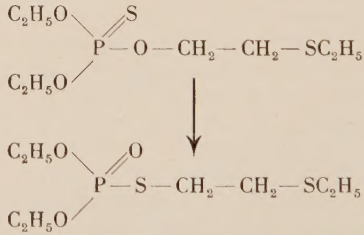
Refraction: $n_D^{18^0} = 1{\cdot}4900$

Molecular weight: 258·1

The volatility of "E 1059", calculated from the vapour pressure graph, amounts to:

15·5 mg/m³ at 20° C
38 mg/m³ at 30° C
96 mg/m³ at 40° C

It has been obverved that the active ingredient transposes at high temperatures. It replaces the sulphur double bonded to the phosphorus by double bonded oxygen:



In this isomeric form, "E 1059" is available as thiolphosphoric ester. Both compounds only differ slightly in their physical behaviour. The physical data are tabulated here for the purpose of comparison:

Table I:

"E 1059"		isomeric "E 1059"	
$n_D^{18^0}$	= 1·4900	$n_D^{18^0}$	= 1·5000
$d_{4^0}^{21^0}$	= 1·119	$d_{4^0}^{21^0}$	= 1·132

Table II:

Vapour pressure: "E 1059"	Boiling point	Vapour pressure: isomeric "E 1059"	Boiling point
0·15 mm Hg =	81° C	0·15 mm Hg =	93° C
0·4 mm Hg =	94° C	0·25 mm Hg =	100° C
0·6 mm Hg =	100° C	0·4 mm Hg =	110° C
2·2 mm Hg =	121° C	0·8 mm Hg =	120° C
3·3 mm Hg =	130° C	1·0 mm Hg =	128° C

Whereas both esters are completely miscible with practically all organic solvents, they differ greatly from each other with respect to their solubility in water:

"E 1059"	isomeric "E 1059"
1 : 15,000	1 : 500

The toxicological data of both compounds are reproduced in Table III, and are compared with those of "E 605":

Table III:

Active substance	Mouse subcutaneously in H_2O + wetter Lethal dose	Rat per os in H_2O + wetter Lethal dose
"E 605"	10—12·5 mg/kg	6·4 mg/kg
"E 1059"	15 mg/kg	75 mg/kg
"E 1059" isomeric	10 mg/kg	2·5 mg/kg

b) The insecticidal properties

According to G. Unterstenhöfer (1952), "Systox" can unfold its toxicity on insects and spider mites both as a vapour and contact poison as well as systemically via the plant. In its action as a vapour and contact poison it can be compared with "E 605" as a polytoxic insecticide. The lethal doses are to some extent higher than in the case of "E 605". In contrast to "E 605", "Systox" does not possess a residual effect on the surface of treated objects.

Due to the power of "Systox" to penetrate into the plant, a far-reaching selective effect can be attributed to it, which is limited essentially to aphids and spider mites.

II. Plant material

In these tests herbaceous plants were chiefly used. Even in the preliminary tests it had been revealed that *Vicia faba* is a particularly favourable object on which to experiment. *Vicia faba,* which can be easily cultivated from uniform seed material in culture solutions, building sand and garden soil, produces in its habitus material of equal value, and can be readily infected with *Doralis fabae* Scop. Therefore, this plant was used in the majority of the tests. Wherever large numbers of woody plants were required for special investigations, *Salix viminalis* were placed in pots in early Spring to take root. Other woody plants used in large numbers were *Pelargonium zonale.* Further plants included in the tests were: *Cyclamen persicum, Cypripedium harrisianum, Humulus lupulus, Mahonia aquifolium, Malus silvestris* varieties "Wealthy" and "Fey's Frühe",

Phaseolus vulgaris, Pirus communis "Pergamotte", *Primula obconica, Rhoeo discolor* and *Tropaeolum majus.*

III. Methods of bioassay

a) Biological test

Since a specific chemical analysis for "Systox" is not known, the active ingredient can only be traced in the biological test, that is to say apart from using radioactive marked molecule. The acknowledged methods of bioassay, with the exception of the aphid test, cannot be carried out on the plant or on isolated parts of the plant. Even in the aphid test, the lowest limit for tracing the active ingredient in the plant is with a "Systox" concentration of 0·001 %. To prove that the measured radioactive material in the order of its quality was just as insecticidally effective, an aphid test was carried out in each series of tests. For the test itself, 50 *Doralis fabae* Scop., *Doralis pomi* de Geer or *Macrosiphon solanifolii* Ashm. were applied with a soft hair brush to each plant part being investigated, dependent in each case upon the type of plant used. Isolated plant parts were tested in Petri dishes, and the number of insects killed was registered after 24 hours.

b) Bioassay with ^{32}P marked molecule

The possibility of tracing quantitatively even the smallest quantities of the preparation exists only in the indication with radioactive elements. This method of bioassay may be regarded as particularly suitable, and in view of the following it can be used without misgiving:

1. Over a period of 8 weeks in the pH range of 3 to 7, the preparation in aqueous solutions does not undergo any autolytic changes which might reduce its toxicity. In the alkaline range at a pH of 8 to 9, the compound saponifies quickly with the result that the preparation loses its toxicity.
   With the exception of the sieve tubes, a degree of acidity of pH 5·6 to 6 is to be expected in the plant. The sap in the sieve tubes, on the other hand, produces an alkaline reaction. This means that it would be possible for the quantities of preparation reaching the phloem to become saponified. Therefore, dependent upon the length of time the active ingredient remains in the phloem, allowance must be made, under certain circumstances, for a loss of toxicity. Such saponification products, however, are included in the measurement due to the content of ^{32}P.
2. According to the toxic values obtained in the biological test on aphids, the compound is apparently stable in the plant. The following test may serve to strengthen this supposition.
   Vicia faba plants, approximately 10 days old and grown in compost soil, were removed from the pots, the soil was washed off the roots, and the plants were placed by their roots in a 0·1 % solution of "Systox" where they were left for 6 hours. Afterwards the roots were washed clean under running water, and the plants were potted again. In the greenhouse they remained toxic to aphids *(Doralis fabae* Scop.) for five weeks.

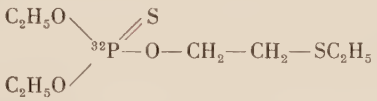
IV. Methods employed with radioactive, marked "E 1059"

a) General

The radioactive preparation was synthesized in the laboratories of G. Schrader*) at the Farbenfabriken Bayer plant in Elberfeld. The marked

*) At this stage I would express my gratitude to Dr. Schrader and Dr. Mühlmann for the synthesis of the preparation.

element is phosphorus and is present in molecule as ^{32}P. For the purpose of the synthesis, the pure P = *S* compound was selected because it guarantees that the substance has a high degree of purity, evaluated in this case at 98 %. The constitution of the molecule was as follows:



The investigations were carried out in accordance with recommendations made in the literature. (Martin and Russel 1950, Schubert 1951.) A contamination of the room had to be prevented at all costs, and, therefore, the following precautions were taken:

1. a) the measurements were taken in a room specially prepared for the purpose;
   b) the application of the preparation and the processing of the material were carried out strictly separate from each other;
2. the hands of the assistants were examined several times daily for contamination;
3. the specimens under examination were only handled with metal or glass instruments;
4. at the end of each stage in the tests the instruments were cleaned thoroughly in water or acetone;
5. the glass vessels were thoroughly rinsed out ten times in water each time they had been used (it was found that, in general, five rinsings sufficed to clear the vessels of preparation);
6. when working with high concentrations of the preparation, the glass countertube and the pipettes were rinsed in acetone.

All the experiments with radioactive material were carried out between Juli 11 and August 22, 1952, in a well ventilated and shaded greenhouse or in a room fitted for the purpose with artificial lighting. The vapour action of the preparation did not have to be allowed for in the arrangement of the tests since untreated check plants, which had been standing amid treated specimens, never showed a measurable deposit of the preparation. Apparently once the preparation has become volatile it is not capable of condensing again at normal temperatures. As a further precaution against the greenhouse becoming infected, all the vessels containing treated plants or parts of same were placed in Petri dishes.

Methodical difficulties in working up the material were only involved insofar as the "fresh weight" had to be taken as the reference unit in the measurement. Mention has already been made that the preparation is readily volatile, and for this reason dry ashing had to be left undone. A suitable method proved to be the processing of the plant substance by means of concentrated H_2SO_4 followed by an oxidation of the carbon with concentrated H_2O_2 until the liquid had been rendered clear as water. Blank tests were carried out to determine the loss of radioactive substance in the ashing process. As the concentration increases, up to 10 % of radioactive material can be written off due to smearing on the glass vessels.

Working process:

1. The plant material, weighed to within ± 0·010 g (maximum weight 1 g, mostly 0·5 g), was placed in a 25 cc Kjeldahl flask, and mixed with 2 ccs of concentrated H_2SO_4. A strong degree of warmth set in as a result of the

momentary extraction of water from the tissue. The flask was well shaken, and placed for 10 minutes in a heated sand bath. After the flask had been shaken again, approximately 15 drops of a Perhydrol solution were added. Once the flask had cooled off, the contents were poured into a 10 cc measuring cylinder and, by repeatedly rinsing the Kjeldahl flask, filled up to 10 ccs. This quantity of liquid was then placed in a 20 cc flask with ground stopper.

2. The measurements were taken in a room with a temperature of $20^0 \pm 1^0$ C. The measuring apparatus was constructed in Dr. Sybertz's physics laboratory at the Farbenfabriken Bayer plant in Leverkusen. It operated with a 32 fold reducer. In order to maintain equal conditions during work with the counter apparatus when measurements were made over a period of 6 weeks, a voltage stabilizer, with a current consumption adjusted to suit the apparatus, was connected in series. The countertube used had a plateau of 1020 volts to 1400 volts. The measurements were taken at a countertube voltage of 1235 v. Thus any possible change in voltage was left without influence upon the rate of counting. Since ^{32}P exhibits a relatively weak β-radiation, the absorption of air is considerably large. It would appear advisable for the radioactive material to be brought into close contact with the countertube jacket. This is easily done with a liquid countertube like the one used in the investigations (Russel et al. 1950). The volume of this countertube is 10 ccs, and for this particular case it was calibrated for measurements of phosphorus. The calibration was done merely to be able to determine the specific activity of the preparation (absolute measurement). All other measurements taken during the course of the test series were relative measurements. Provided the volume in the fluid countertube is kept constant at 10 ccs, the corrections for the geometric factor and, in the case of relative measurements, for absorption and autoabsorption are eliminated. The count of the specimens was carried out up to 1000 impulses, at low concentrations over a period of 10 minutes. Before each reading of activity was taken, the zero effect was determined for five minutes.

Evaluation of the readings:

In the formulation of the result of a measurement, the following factors have to be taken into consideration (Schubert 1951):

a) the dissolving power of the apparatus;
b) the zero effect;
c) the drop in intensity with time.

With respect to the dissolving power of the apparatus, the following must be considered. When statistically distributed impulses are counted, such as they occur in all measurements of activity, insufficient discrimination of the registration device can prove to be most disturbing. That applies particularly to large numbers of particles. Should a particle follow the preceding one at an interval shorter than the so-called period of dissolution τ, it will not be registered because the discharge in the countertube is not broken down. The correction formula reads

$$N_0 = \frac{N}{1 - N \cdot \tau}$$

whereby τ equals 10^{-5} for the measuring device referred to in this case.

After the time of dissolution has been determined and the result of the measurement has been corrected, the zero effect should be subtracted from the

rate of impulse. Its size depends upon the type of countertube used, and results from the cosmic-ray radiation and the radioactive traces of air.

Regarding the drop in intensity with time, the following points should be made. If the factors "a" and "b" are considered in the result of the measurement, then the conversion must be made to the date the test was started. If "N_0" represents the number of registered impulses per minute at the start of the test (initial activity), then the activity after the time "t" is only

$$N = N_0 \cdot e^{-\lambda t} = N_0\, e^{-(\ln 2)\frac{t}{T}}$$

whereby λ is the constant of radioactive transformation or T represents the half-value life of the isotope. Accordingly the correction factor amounts to

$$N_0 = N \cdot e^{(0{\cdot}693\,\frac{t}{T})}$$

and for ^{32}P at a half-value life of 14·295 it is

$$N_0 = N \cdot e^{(0{\cdot}0485 \cdot t)}$$

In all the given results, these three factors have been corrected. Furthermore all the values have been guaranteed according to the formula for statistically distributed impulses $= n \pm \sqrt{n}$.

b) Quantitative determination of the preparation

Approximately 20 g of radioactive material were available for the investigations. A solution of 0·05 %, the concentration mostly used, had a specific activity of $\approx 4{\cdot}5\,\mu$C in the litre. Plants treated with 0·1 or 0·05 % solutions, irrespective of whether they were watered or sprayed, on no occasion exhibited perceptible injuries. Thus it may be concluded that the growth of the plants was not impaired by the radiation of the preparation.

The content of preparation had to be determined in the plant on the basis of the measured activity. As already mentioned, all the other measurements, with the exception of the above activity determinations, were recorded relatively. For these, it is necessary to compile a calibration table and to plot a calibration graph since, by measuring out a standard solution in different concentrations, it is possible to determine the exact quantity of preparation in weight for each number of impulses recorded.

At this stage it must be pointed out once again that all the following quantitative data on the preparation content in plant material are computed solely from the activity determinations. The employed method did not directly indicate the presence of active ingredient but merely the radioactive phosphorus atom.

When active ingredient or "E 1059" is mentioned in this paper, only the diethyl thionophosphoric ester of β-oxyethyl thioethyl ether is meant, whereas "Systox" or preparation refers to the active ingredient — emulsifier ratio 50 : 50.

From the radioactive marked "E 1059", a 1 % by volume "Systox" solution made up of

1 cc ± 0·001 cc active ingredient
1 cc ± 0·05 cc emulsifier
198 ccs ± 0·05 cc Aqua dest.

was prepared, followed by dilutions ranging from 0·7, 0·5, 0·3, 0·1, 0·07, 0·05 % etc. to 0·000 001 %. Each concentration marked in Table IV with an asterisk was measured 10 times in the course of 14 days. The corresponding average values are recorded in Table IV and Fig. 1.

The preparation could be determined theoretically and be perceived up to a dilution of 10^{-9}, and at 10^{-8} it could be measured exactly. The numbers of impulses allowed for theoretically were considerably higher than values actually measured. The differences are listed separately at the end of Table IV.

Table IV: Standard measurements of radioactive marked "Systox" solutions with 10 ccs liquid ("E 1059" $= d\,\frac{21^{\circ}}{D}$ 1·119)

Impulses/Min.	"Systox" concentration	Active ingredient in mg
21 292	0·01 %	0·559 500
19 164	0·009 %	0·503 550
17 036	0·008 %	0·447 600
14 908	0·007 %	0,391 650
12 780	0·006 %	0·335 700
10 662	0·005 %	0,279 750
8 534	0·004 %	0·223 800
6 406	0·003 %	0·167 850
4 278	0·002 %	0,111 900
2 150	0·001 % *)	0·055 950
1 937	0·000 9 %	0·050 355
1 724	0·000 8 %	0·044 760
1 512	0·000 7 % *)	0·039 165
1 299	0·000 6 %	0·033 570
1 086	0·000 5 % *)	0·027 975
873	0·000 4 %	0·022 380
660	0·000 3 % *)	0·016 785
448	0·000 2 %	0·011 190
235	0·000 1 % *)	0·005 595
214	0·000 09 %	0·005 036
192	0·000 08 %	0·004 476
171	0·000 07 % *)	0·003 917
150	0·000 06 %	0·003 357
128	0·000 05 % *)	0·002 798
107	0·000 04 %	0·002 238
86	0·000 03 % *)	0·001 679
65	0·000 02 %	0·001 119
43	0·000 01 % *)	0·000 560

Values which are lower than these concentrations cannot be measured with the liquid countertube in the order of magnitude allowed for theoretically.

The theoretical values read:

33 Imp./min.	0·000 005 %	0·000 280 mg
24 „ „	0·000 001 %	0·000 056 „
20 „ „	0·000 000 1 %	0·000 006 „

The values actually measured amount to:

20 Imp./min.	0·000 005 % *)	0·000 280 mg
12 „ „	0·000 001 % *)	0·000 056 „

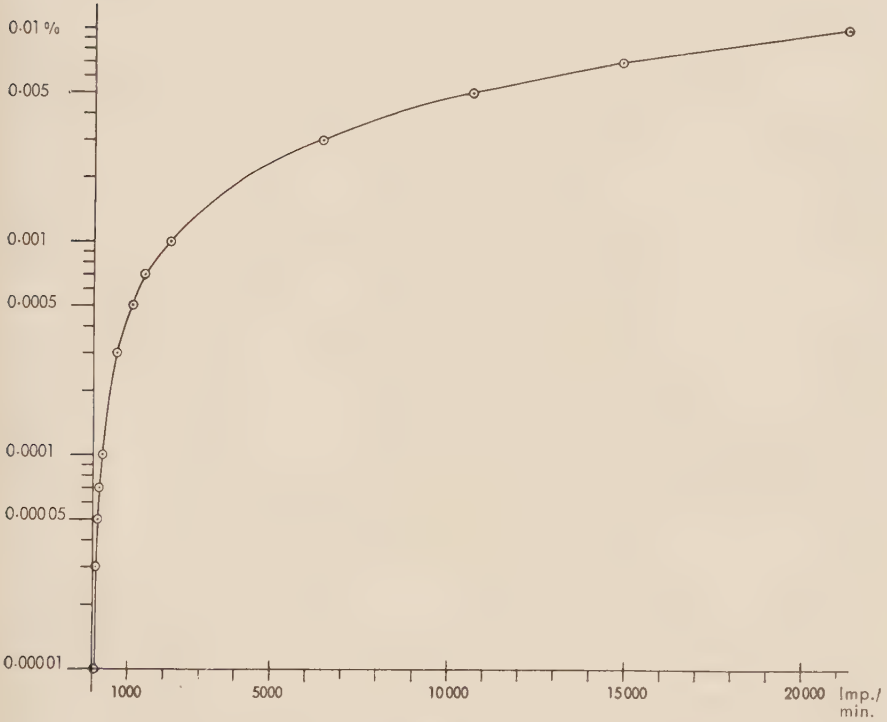


Fig. 1. The measured numbers of impulses for the different concentrations of the ^{32}P marked "Systox" standard solution.

c) Autoradiographs

Whereas the measuring method with the Geiger-Müller counter permits the total quantity of radioactive substance present in a plant part to be determined, it is possible with the aid of the autoradiograph to ascertain the sites in tissues or even in cells where "E 1059" is located. With varying degrees of blackening, the differences in concentration can be established (Blank 1951, Bogoroch 1951, Russel 1949). Unfortunately, considerable difficulties are attached to the preparation of the tissue. Plant material treated with radioactive marked active ingredient would suffer a great loss of activity from an application of the conventional fixing agent and the subsequent imbedding in paraffin (unavoidable

alcohol stages and benzene). Merely because of the lipide-solubility of the active ingredient, a strong extraction occurs in the alcohol series and in the benzene. The treatment too takes 4 to 5 days until the section is ready when employing the paraffin imbedding technique. For these reasons it was necessary to evolve a special method in order to make the section preparation. In accordance with the method employed by B l a n k (1951), the conventional fixation was dispensed with, and instead a pretreatment was carried out with low-cooled ethylene glycol and an imbedding in "Syntha" wax. This method permitted section preparations to be made within the short time of 12 hours. The losses in activity did not amount to more than 2 % for the whole imbedding process, whereas check losses in activity in fixations in Bouin and Carnoy amounted to 45 and 50 %.

The single stages of preparation were as follows:

The plant objects, in this case leaves, following the imbibition with the active ingredient, were cut into sections approximately 10 mm^2 in size and deaerated in water for a short period by means of a water jet pump. A mixture of common salt and ice was prepared in a Dewar vessel, and a vessel containing 20 ccs of ethylene glycol cooled to —20° C. The objects were inserted in the low-cooled ethylene glycol where they were left for 3 hours. Subsequently the ethylene glycol was heated up gradually to room temperature, and the plant parts were transferred to a 1 : 1 mixture of ethylene glycol and "Syntha" wax with a temperature of approximately 30° C, where they were left for 2 hours at 50° C in the warming cabinet. The next stage comprised the use of pure "Syntha" wax, and lasted 6 hours. Afterwards the objects were imbedded, and cut on the microtome at an adjustment of 10 μ and 15 μ. The serial sections were placed on slides which had been prepared beforehand in a solution containing 1000 ccs Aqua dest., 5 g gelatin and 0·5 g chromalaun. Slides treated in this manner can be stored indefinitely. The sections were expanded by warming the slides, and the "Syntha" wax melted. Afterwards they were allowed to cool again and a well moistened strip of filter paper was placed on top. The filter paper was moistened with a 10 % "Syntha" wax solution in thymol saturated aqua dest. Then the filter paper strip was weighted with a second carrying glass. Before the whole assembly was moved to the warming table to dry, slight pressure was applied to the second carrying glass to ensure that there was no air left between the section and the slide. The preparation was dried for approximately 1 to $1^1/_2$ hours at 30° C. At the end of this time the filter paper can be raised slightly and the sections remain firmly in position. The preparation was now ready to be mounted with the stripping film. This film consists of a glass plate, serving as the base, on which a gelatin layer, approximately 10 μ thick, is poured. On top of this layer there is a film emulsion 5 μ thick. The film material must be processed in dark-red light. A piece was cut out of the film material with a razor blade, so large that it projected over the slide with the mounted section both in the length and the width. The razor blade was inserted under one of the cut corners of the film thus enabling the selected surface to be raised. Beforehand a shallow dish, filled to a level of approximately 3 cm with filtered aqua dest., was placed in readiness. The removed film strip was placed on the water surface with the sensitive layer facing downwards. The film must be allowed to swell for 2 to 3 minutes. A prepared slide was then passed under the floating film strip and it was lifted out of the water by the carrying glass. Care had to be taken that there were no air bubbles between the film and the slide. It may be necessary to increase the preparation again, and to drain the water at an inclination of approximately 30°. The three edges of the film strip protruding over the carrying glass were turned down, pulled tight and pressed against the bottom of the slide. The film

was dried with a hot-air blower set at "moderate", the slide was placed safe to light in a black-lined container, and the section was exposed for a half-life period. The time of exposure for ^{32}P is accordingly 14 days. The temperature at the site of exposure should not exceed 20° C. From the 10th day onwards, for the purpose of keeping a check, a slide with mounted film was developed daily, fixed, and the advanced degree of blackening observed under the microscope. The autoradiographs illustrated on pages 43 and 44 were exposed for 12 days. A detailed description of the method has already been given in another paper (Tietz 1952). Macroscopic general views are autoradiographed in such a manner that treated leaves are simply placed on an X-ray film which carries cellophane paper between the leaf and the film foil as a protection against moisture transpired from the leaf. The time of exposure for these autoradiographs is only 10 days. The X-ray film material must be processed in complete darkness.

E. The absorption of radioactive marked "E 1059" through the intact roots and the upward movement to shoot parts above ground

I. Absorption through the intact root from different media

In this chapter it will be shown to what extent the root absorbs "E 1059" from a culture solution and from sand or from a specified culture soil. The quantity of active ingredient present in the shoot was taken as the measurement for consumption.

a) Culture solution

For the studies on absorbability from a culture solution, *Vicia faba* plants cultivated in sand, 14 days old and about 30 cm long, were removed from the pots and freed from clinging sand approximately 5 hours before they were tested. They were then placed by the roots in water and left there until the test began. At the start of the experiment they were transferred to a nutrient solution containing a 0·05 % concentration of radioactive marked "Systox". 100 cc wide-necked Erlenmeyer flasks were used as culture vessels. The plants stood exposed in 100 ccs of solution. The vessels were not additionally aired. The reason for this was to prevent large quantities of preparation being dispersed from the culture solution by an air current. It was possible to keep the plants in this solution until the third day of the test, after which the first injuries became perceptible. Apart from a slight brown colouring of the roots in the initial stages, necrosis appeared simultaneously on the peripheries of the middle leaves. On the 4th day the test had to be abandoned since the injuries had become so severe that it no longer appeared advisable to evaluate these plants. The roots had meanwhile assumed a dark colour, and the leaves had withered. The quantitative absorption through the *Vicia faba* root is illustrated by curve A in Fig. 2.

b) Sand and culture soil

In a parallel test, *Vicia faba* plants, which at an age of 14 days were already 35 cm long (measured from the hypocotyl to the shoot apex), were also treated with radioactive marked "Systox". The plants were cultivated in washed sand which was watered daily with nutrient solution, also in normal compost soil. The pots had a volume of 400 ccs. The potted plants could only be dosed with 50 ccs of the same "Systox" solution since, if 100 ccs of solution had been

applied, the greater part would have run to the bottom of the pots. On the other hand, it was not possible to reduce the volume to 50 ccs in the test series in which the roots were supposed to absorb the active ingredient from the culture solution, otherwise only a small proportion of the root system would have been washed by the solution. Therefore, allowances had to be made for differences in the absolute quantity of active ingredient offered to the roots. Apart from this, a further dilution of the offered preparation was suffered by the potted plants as a result of the 50 ccs of water applied to them daily. For this reason an absolute comparison cannot be drawn between curve A in Fig. 2 and curves B and C which show the absorption of active ingredient from sand and culture soil respectively.

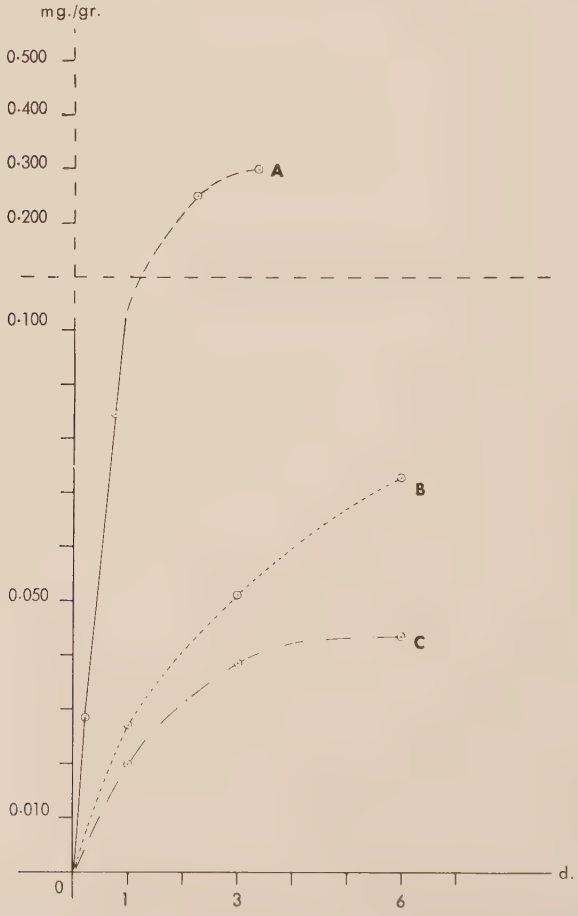


Fig. 2. Content of active ingredient measured in the shoot part (*Vicia faba* plants) growing above ground, during the course of 6 days. The active ingredient was absorbed by the root from different media.

A = from a solution (moving linearly)
B = from sand
C = from soil

The values given in Fig. 2 have been obtained from each of 3 parallel tests. As the curves show, there is a perceptible difference between each of the three media. The absorption from a culture solution occurs most rapidly, the active ingredient penetrates into the plant linearly, and a high concentration is attained. In building sand it is a much slower process, whereby the quantity absorbed here is only a quarter of that absorbed from the culture solution. The slowest rate of absorption with the lowest consumption is in plants cultivated in culture soil. From the injuries it is also evident that the compatibility limit for *Vicia faba* is exceeded at 0·250 mg active ingredient per gramme plant material. In sand and soil the absorption of "E 1059" concentrations is between 0·030 and 0·080 mg/g plant. In the main, it is only the media themselves which can be held responsible for the differences. Although the sand curve (B) lies above the culture soil curve (C), the total quantity of active ingredient absorbed by *Vicia faba* plants cultivated in sand is no greater than that absorbed by the plants cultivated in culture soil. That is proved when the ratio of concentration in the individual organs is taken into consideration. The curves in Fig. 3 provide a picture of the movements of concentration which occur in the various organs during the course of 12 days.

The root absorbs the active ingredient freely from a culture solution. The concentration rises linearly until the tissue is totally damaged. In plants cultivated in sand, the active ingredient content in the root continues to increase until the 3rd day (see Fig. 3, A II). From then onwards until the 6th day there is a rapid decrease in the content of "E 1059", which continues, although more gradually, until the 12th day. Apparently three applications of nutrient solution totalling 150 ccs are sufficient to wash most of the active ingredient out of the building sand, with the result that no further quantities penetrated the root from outside. In order not to interrupt the tests it was not possible, while they were being carried out, to examine the building sand for its content of active ingredient.

When the total concentration in all three organs is calculated, namely the root, the shoot axis and the leaves, based on the total weight of the plant, an increase of active ingredient in the plant amounting to approximately 50 % may be determined from the 1st to the 3rd day. From the 3rd to the 12th day, however, there is a slight decrease of about 9 %. This loss cannot be discussed at this stage. It can merely be established that the active ingredient is not stored in the root for very long but is moved in the transpiration stream to those parts of the plant above ground. In conformity with the decrease in "E 1059" in the root, there is an accumulation in the organs growing above the surface. Since there is no longer a depot of preparation in sand, the curves in Fig. 3, B II and C II, only show a slight increase in active ingredient from the 6th day onwards.

The position is quite different in the tests where applications were made in culture soil. The soil is apparently able to retain the active ingredient because on the 1st day the quantity absorbed is only about half that absorbed from building sand (see Fig. 3, A II and III). The increase of concentration in the root is most constant up to the 6th day, but from then until the 12th day the curve drops slowly. This would indicate that the depot of preparation in the soil, although it is still present, is no longer very large, otherwise there would be a sharp drop similar to that illustrated in the curve of Fig. 3, A II. The content of active ingredient in the whole plant increases constantly from the 1st until the 12th day, and the translocation to the organs above the ground from the 6th day onwards is quite considerable

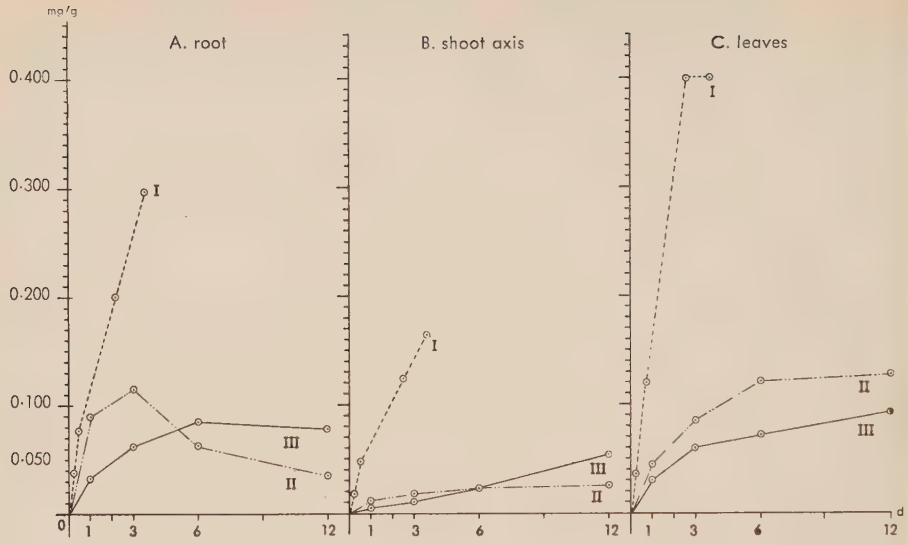


Fig. 3. Concentration of active ingredient in the various organs of *Vicia faba* plants when absorbed by the root from different media, in the course of 12 days.
I = from a solution
II = from sand
III = from soil

II. Reaction of the root in a solution of active ingredient

The measurements for the absorption of "E 1059" showed that roots of the plants react differently dependent upon the type of culture medium surrounding them. It, therefore, appeared suggestive to examine how the root itself reacts to a solution of active ingredient. The results obtained admittedly were not sufficient in themselves to explain the regularities expounded in the preceding chapter but they did illustrate an interesting reaction on the part of the root. In view of this the following descriptions are given at this point:

a) Increased water absorption by plants in a solution

Plants of *Phaseolus vulgaris*, which were cultivated in building sand and had just completed development of the primary leaves, were removed from the pots 6 hours before the start of the test and placed in a nutrient solution. Afterwards one plant was placed in a nutrient solution containing an addition of 0·01 % "Systox", whereas a second was left in the nutrient solution as a check. It had to be established whether there would be any change in the absorption of water by the root due to the presence of active ingredient. The measurements were taken in potentiometers which were placed in a light thermostat at an illumination of approximately 5000 lux and at a permanent temperature of 22° C ± 1° C. The relative humidity was 65 %. It was observed that the roots immersed in a nutrient solution with an addition of 0·01 % "Systox" absorbed far more water than the checks. After 5 minutes the root of the treated plant appeared to be far more permeable than that of the check. When two hours had elapsed the maximum was reached, whereby the absorption of water in the case of the plant to which active ingredient had been

applied had increased by 35 %. During the next few hours this effect slowly receded until between the 8th and the 9th hour the quantity absorbed was the same as that in the check. In fact during the next four hours it was slightly under the value obtained in the check test, although both values were again equal by about the 15th hour (cf Fig. 4).

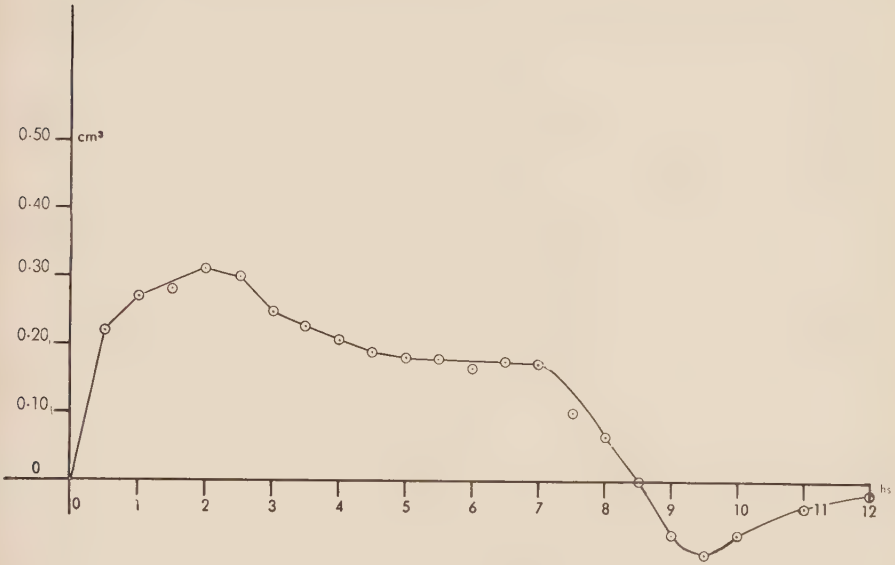


Fig. 4. Increased water absorption by *Phaseolus vulgaris* from a 0·01 % "Systox" solution, through the root. The abscissa represents the linear water absorption of the check plant.

b) *Ratio of concentration in a solution*

In order to examine more closely the absorption or mechanism of penetration of the active ingredient, the level of concentration of the radioactive substance in a culture solution was followed for 6 days (after D a v i d , 1951). During this period the plant had constantly absorbed water from the nutrient solution. 10 day old *Vicia faba* plants, which were cultivated in sand, were used as test objects.

This time lower concentrations of preparation in the nutrient solutions had to be selected because of the injuries inflicted upon the roots on the 3rd day. Therefore, one test series was carried out with 0·01 % radioactive marked "Systox" and a second with 0·005 %, each with three replications. In this way, namely due to the considerable reduction of the concentrations, it was possible to continue the tests until the 6th day. The first injuries to the roots occurred on the 4th day, gradually becoming worse until the 6th day. Just as in the tests described on page 15, no artificially produced air was supplied in order not to disperse the preparation from the nutrient solution by air current. The plants were placed in 100 cc wide-necked Erlenmeyer flasks, and dosed with 100 ccs of nutrient solution with a content of radioactive marked "Systox" of 0·01 % or 0·005 %. In each test series there was a check flask containing no plants. Cellulose was wound around the plants between the hypocotyl and a pinnate leaf to serve as a seal. A similar seal made of cellulose was fitted to the check flasks. This prevented large quantities of radioactive preparation evaporating from the nutrient solution.

The plants were removed before each extraction of liquid, the adhering water was collected, the culture solution shaken, the remaining volume ascertained, and 10 ccs pipetted off to determine the activity. Following the measurement, the liquid was again added to the solution.

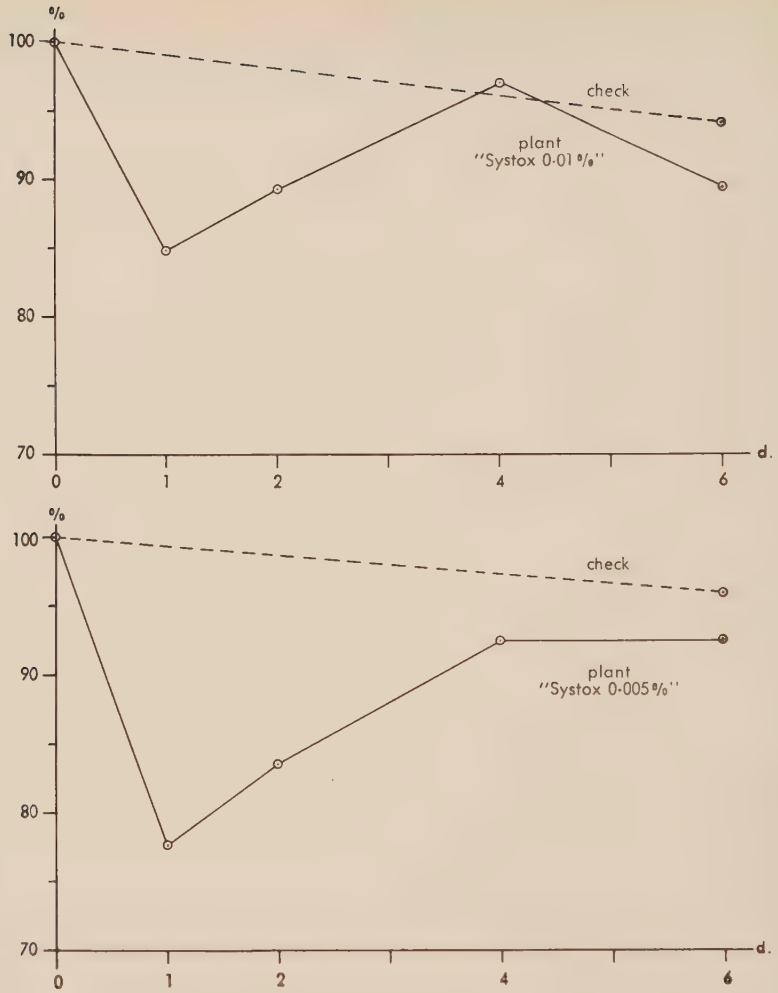


Fig. 5. Concentration changes of active ingredient content in a nutrient solution during the course of 6 days, with respect to the absorption of water by *Vicia faba* plants through the roots.

The results obtained are reproduced in the graph in Fig. 5. The points drawn in the curves represent the average values from three measurements. After one day there is a large drop in the concentration of the preparation. Much more active ingredient is absorbed by the roots than would correspond to the quantity of liquid consumed. During the next three days the concentration in the remaining solution increases again. Altogether the results show that the root absorbed the active ingredient preferably during the course of the first day. Afterwards the power of absorption returns to a more or less constant, standard value which corresponds to the volume of the water absorption.

c) Recretion phenomena in the root

In the preceding chapter it was explained that within four days the *Vicia faba* root undergoes a considerable change with respect to its absorbability. Based on the relative increase in the content of active ingredient present in the culture solution during the 2nd to the 4th day, it may be assumed that the root can recrete "E 1059". To check this, *Vicia faba* plants cultivated in sand were removed from the pots, and placed by their roots in a 0·1 % solution of radioactive preparation where they were left for 6 hours. The roots were then washed clean under running water, and the absorbed quantity in 1 g root determined. It amounted to an average of 0·045 mg/g. Afterwards the roots of the remaining plants were left in slowly running water for 6 hours, and then for a further 6 hours in Erlenmeyer flasks containing 100 ccs of water. During this period the roots had again recreted considerable quantities of preparation even though they had discharged $^2/_3$ of their original concentration of active ingredient in the running water. The recretion in this case reaches its maximum (after 36 hours) at 0·001 1 mg active ingredient per cc water (residual solution). At this stage there is still 0·010 6 mg active ingredient in one gramme of root. This means that in this case the root concentration still does not correspond to outside concentration. Such balance is not established at all. The root admittedly recretes "E 1059" but, on the other hand, so much active ingredient is retained that the concentration value of the solution is permanently low. The exact values are reproduced in Table V, and plotted in the graph in Fig. 6.

Table V: The exudation of active ingredient from the intact root in a Knop's nutrient solution (over a period of 60 hours) with respect to *Vicia faba* plants which, before the start of the test, had absorbed a 0·1 % "Systox" solution through the roots for 6 hours.

Plants	Time and test	Weight of root in g	Content of active ingredient in the root total mg	mg/g	Residual quantity of nutrient solution in ccs	Exudated quantity of active ingredient in the residual solution expressed in mg	Quantity of active ingredient in 1 cc of residual solution expressed in mg
1	6 hrs. imbibition, then ashed	4·200	0·222 4	0·052 5	—	—	—
2	" "	3·850	0·130 5	0·033 9	—	—	—
	Average of 1 and 2	4·025	0·176 4	0·043 2	—	—	—
3	After 12 hrs., 6 hrs. of which washed in running water	3·520	0·070 0	0·019 7	98·5	0·018 5	0·000 19
4		4·550	0·048 3	0·010 2	99·0	0·016 3	0·000 16
	Average of 3 and 4	4·035	0·059 2	0·014 9	98·7	0·017 4	0·000 17
5	After 36 hrs.	4·300	0·055 7	0·012 5	90·5	0·101 6	0·001 12
6	" "	3·120	0·028 0	0·008 6	92·5	0·092 0	0·001 0
	Average of 5 and 6	3·710	0·041 9	0·010 6	91·5	0·096 8	0·001 1
7	After 60 hrs.	3·720	0·041 3	0·010 7	80·0	0·029 5	0·000 37
8	" "	4·680	0·045 1	0·009 6	80·0	0·044 8	0·000 56
	Average of 7 and 8	4·200	0·043 2	0·010 2	80·0	0·037 1	0·000 47

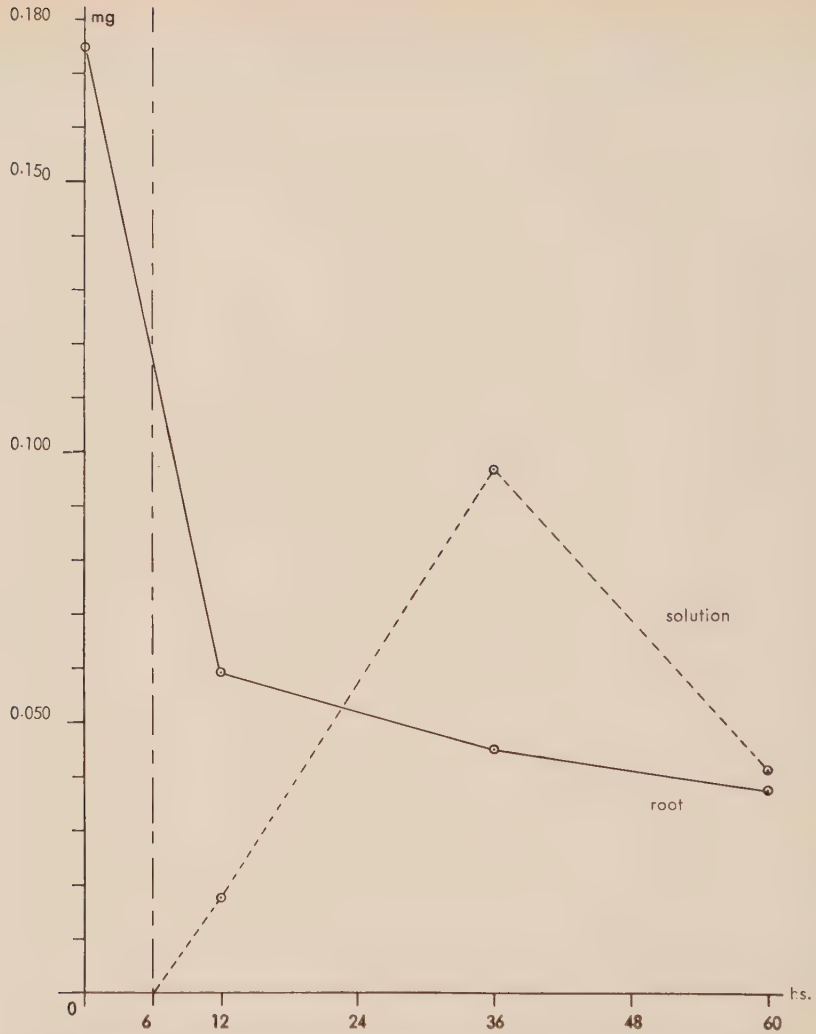


Fig. 6. Recretion of radioactive marked preparation through the intact root in a nutrient solution, and the accumulation of active ingredient in same.

III. Upward movement of "E 1059" in the plant

For the tests which have been described it was evident that the active ingredient, as soon as it had been absorbed by the root, was immediately translocated to those parts of the plant growing above the ground. With the aid of an air countertube, it was possible to determine the speed of movement in the shoot for *Vicia faba* and *Salix viminalis:*

Plants were removed from the pots, placed in water, and two hours later transferred to a 0·1 % solution of radioactive marked "Systox". The countertube was clamped at a

specified level, and the leaves of the plants were fixed next to it. The counter mechanism of the recording device was kept under constant supervision. In this manner, it was possible to determine exactly the first rate of impulse exceeding the level of the zero effect (including the statistic error of $n \pm \sqrt{n}$).

The result was a speed of movement for *Vicia faba* of approximately 3·2 metre/h at a temperature of 21° C. The relative humidity amounted to 60 %, and the intensity of illumination 10,000 lux. The speed of movement for *Salix viminalis* was measured under the same environmental conditions. In this case, however, the experiment was extended insofar as the speed was first measured in still air, as for *Vicia faba*, and secondly in a strong current of air (produced by means of a fan). The speed of movement of the active ingredient in still air amounted to 80 to 90 cm/h, and to 1·20 metre/h for turbulent air. This would indicate that the translocation of "E 1059" in the shoot takes places primarily in the xylem with the transpiration stream.

A perusal of Table VI (explanation of text given in Fig. 7) will show that in the first instance the shoot axis only serves as a conducting organ. It is not until after 24 hours have elapsed that the content of active ingredient in the axis tissue gradually increases. Within this period the leaves have absorbed three

Fig. 7. The different organs of the *Vicia faba* plant.

times as much "E 1059". Furthermore there is a definite drop in concentration in the shoot axis, and in the region of the shoot end the content of active ingredient decreases. On occasions, however, a damming up may be observed at the apex.

Table VI: The distribution of active ingredient in the different organs of *Vicia faba* plants when absorbed from sand and soil in the course of 12 days. (The figures marked with asterisks refer to newly developed organs.)

Plant organ	after 1 day		after 3 days		after 6 days		after 12 days	
	Sand mg/g	Soil mg/g	Sand mg/g	Soil mg/g	Sand mg/g	Soil mg/g	Sand mg/g	Soil mg/g
Tip	0·017 9	0·019 5	0·021 3*	0·032 8*	0·048 3*	0·034 7*	0·042 3*	0·118 6*
6th leaf	0·016 7	—	0·076 2	—	0·095 4	—	0·083 0	0·085 8*
5th leaf	0·053 2	—	0·080 2	0·097 7*	0·118 6	0.102 7*	0·129 8	0·117 4*
4th leaf	0·049 2	—	0·110 6	0·064 4	0·153 0	0·074 5	0·165 4	0·076 2
3rd leaf	0·052 6	0·041 2	0·112 5	0·073 4	0·156 5	0·071 7	0·175 4	0·091 5
2nd leaf	0·061 1	0·038 1	0·111 8	0·058 1	0·166 5	0·065 5	0·146 8	0·084 1
1st leaf	0·054 4	0·035 5	0·079 0	0·059 8	0·110 7	0·056 5	0·139 4	0·083 0
Shoot axis								
„ „ apical	0·006 8	0·005 8	0·014 1	0·017 2	0·021 1	0·015 4	0·016 2	0·032 6
„ „ centre	0·011 3	0·005 2	0·018 9	0·013 2	0·020 6	0·023 7	0·025 7	0·059 6
„ „ basal	0·014 5	0·007 3	0·024 1	0·021 2	0·025 6	0·028 8	0·030 8	0·071 1
Root	0·091 6	0·033 0	0·115 2	0·061 5	0·062 1	0·084 5	0·035 6	0·079 0

Previous observations showed that a transverse diffusion occurs in the shoot axis. In order to investigate this, woody plants had to be selected on which the bast can be easily removed from the wood. The willow *(Salix viminalis)* is suited to this purpose. Sets cultivated in a corresponding manner were removed from the pots and placed by the roots in a 0·1 % solution of radioactive marked "Systox".

The sets were placed 1 cm above the liquid surface in the shape of a ring. Two hours later it was possible to trace as much as 0·011 3 mg radioactive marked active ingredient per gramme tissue in the secondary bark. In order to eliminate any possible upward movement in the phloem, in the next test a ring 1 cm wide was formed above the liquid level with a second one 10 cm above it. This intermediate piece of secondary bark again showed, after the same period of time, 0·010 mg radioactive substance per gramme tissue.

This should prove that the active ingredient, at the respective level reached by it, passed from the wood part into the bark. It has, however, not been clarified whether it is only present in the bast in an amount corresponding to the concentration in the water supplying the tissue or whether it reached there primarily

as a result of its lipide-solubility. Table VII gives the results of tests in which willow sets were subjected to a 0·1 % "Systox" concentration for 1 and 8 hours. It shows that the active ingredient is stored in the bark.

Table VII: The distribution of radioactive marked active ingredient in wood, bark and leaf of *Salix viminalis* following absorption through the root from a solution.

Time in hrs.	Main shoot		Rate: Wood to bark	Lateral twig		Rate: Wood to bark	Leaves
	Wood mg/g	Bark mg/g		Wood mg/g	Bark mg/g		mg/g
1	0·008 8	0·001 2	7·33	0·004 2	0·000 8	5·25	0·004 7
8	0·012 6	0·007 6	1·65	0·018 8	0·004 9	3·83	0·022 5

Table VIII gives the results obtained with 3 sets placed in culture soil which 48 hours beforehand had been watered with 100 ccs of a 0·1 % "Systox" solution. The results conform fundamentally with those of the preceding test. A new factor discovered in this case was that the bark tissues of the main shoot showed a tendency to store the active ingredient in larger quantities. This phenomenon is not so pronounced in the lateral twigs.

Table VIII:

Plant No.	Content of active ingredient: Main shoot		Rate: Wood to bark	Lateral twig		Rate: Wood to bark	Leaves
	Wood mg/g	Bark mg/g		Wood mg/g	Bark mg/g		mg/g
1	0·013 8	0·017 7	0·77	0·017 3	0·011 3	1·53	0·034 2
2	0·014 1	0·019 8	0·71	0·012 0	0·005 9	2·03	0·030 0
3	0·017 2	0·020 3	0·84	0·025 3	0·013 6	1·86	0·060 0

IV. Accumulation in the leaf after absorption from a solution

A rooted *Vicia faba* plant was subjected to a 0·1 % solution of radioactive "Systox" for 12 hours, and then placed in water for a further 12 hours. Subsequently the content of active ingredient present in the 1 cm wide periphery of the leaf was determined and compared with that in the remainder of the leaf. Similar tests were carried out with cut twigs of *Pirus communis*. The results are given in Table IX.

Table IX: Distribution of radioactive marked active ingredient in the leaf following absorption by shoot suction.

Leaf part	*Vicia faba* mg/g	*Pirus communis* mg/g
Leaf periphery, 1 cm wide	0·038	0·220
Leaf centre	0·017	0·130

In both cases, the concentration in the periphery of the leaf is higher than in the centre. This phenomenon can again only be caused by the transpiration stream. The damming i. e. the accumulation of active ingredient in the vessels in the region of the periphery is also made manifest in that the first damage to leaves of plants which have been given a phytotoxic dose appears in the form

Fig. 8. Typical peripheral necrosis following an overdosage of "Systox". The preparation is absorbed through the roots. Object: *Solanum lycopersicum*.

of peripheral necrosis. Fig. 8 shows a section of the pinna of *Solanum lycopersicum*. *Solanum l.* is extremely sensitive, and injuries are already perceptible after a dosage of 0·1 %. The application was made with 100 ccs of solution to the roots. We examined the damage somewhat more closely on another object. For this purpose twigs of *Pirus communis* were cut off under water, and placed in a 0·5 % solution of radioactive marked "Systox" where they were left for 9 hours. 24 hours later the expected injuries had appeared. Fig. 9a shows the adaxial surface and Fig. 9b the abaxial surface of the leaf. The dark brown colour of the peri-

a) Adaxial surface of leaf.

b) Abaxial surface of leaf.

c) Autoradiograph of the leaf illustrated above. The brightest parts are the sites of the greatest accumulation of preparation.

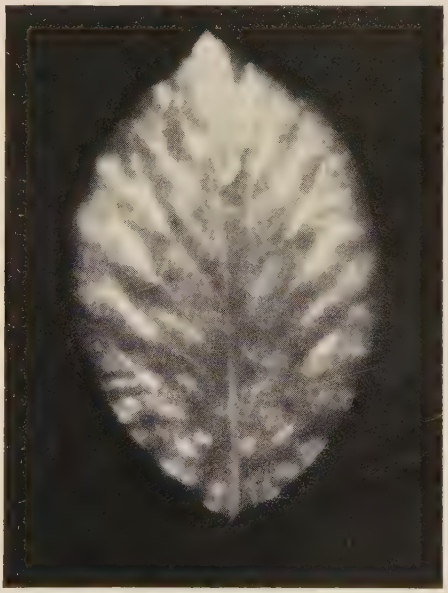


Fig. 9. Necrosis in *Pirus communis* following an overdosage with radioactive marked "E 1059". The active ingredient was absorbed through the cut surface of a twig cut off under water.

phery is immediately detectable on the natural object. The areas between the veins are also dark brown. On the abaxial surface of the leaf it may also be seen that the veins in the region of the periphery are also strongly discoloured. An autoradiograph, in which the same leaf was pressed on an X-ray film, shows particularly high concentrations at the ends of the lateral nerves (cf Fig. 9c).

V. Discussion of the results

The difference in the extent to which "E 1059" is absorbed by the root from different media may be attributed to two factors. Firstly the active ingredient, in its particular affinity to substances with active surfaces, is bonded to the respective substrate. The more active surfaces are present, the less will be the absorbability. Therefore, the root freely absorbs „E 1059" from a solution, to a lesser degree from pure sand, and even less from soil rich in colloid. A comparison of different types of soil can only be made with restrictions. The dilution factor differs dependent upon whether the same quantity of active ingredient is added to a sand or a culture soil. Accordingly, exact statements can only be made when the plants are placed in a culture solution.

Under such conditions, the great ability of the root bark to store active ingredient is most striking. After a short period, a far higher concentration of active ingredient is found in the root than in the shoot above ground. Finally, the concentration in the root is greater than that in the outside solution.

Apparently the finding can only be explained by the ultrafilter-lipoid theory (Ruhland 1909, 1912, 1914; Höber 1926; Gellhorn 1929; Collander 1933; Schmidt, W. J., 1939; Ruhland and Heilmann 1951). The reason for the preferred absorption of the "E 1059" molecules may be found, therefore, in the lipide-solubility of the substance. On penetrating the wall of the root hairs, the "E 1059" molecules are absorbed by the lipoids present, whereas, with respect to the water absorbability, only the wall pores are penetrable.

Roots of *Phaseolus* plants immersed in "Systox" solutions are far more permeable than roots placed in a check solution containing no active ingredient. The increased absorption of water reaches its maximum (35 %) in the 2nd to the 3rd hour.

The assumption that respiration is intensified is the most probable explanation for the increased permeability. It is regrettable that no facts have come to light with respect to the influence exerted by "E 1059" on the process of respiration. It would also be thought that the active ingredient causes a change in the structure of the wall.

The movement of "E 1059" in the shoot axis proceeds in the same manner as that of the nutrient salts. Here a lateral diffusion may be established although this is kept to within moderate limits. The concentration in the parenchymatous tissue, in the bast of woody plants, increases slowly. The active ingredient in the conducting water is, therefore, moved primarily in the xylem of the shoot axis and the leaves. These findings are in complete agreement with the results obtained by Stout and Hoagland (1939) for the movement of salt in plants as indicated by the radioactive isotopes of phosphorus absorbed by roots.

The increased accumulation of active ingredient in the peripheral zones of leaves need cause no surprise. It can be explained quite simply by the course of the transpiration stream and its blind termination in the parenchyma.

F. The penetration of radioactive marked "E 1059" following application to the leaves

I. Quantities of active ingredient penetrating into the leaves and the speed of penetration

14 day old *Vicia faba* plants, which had been cultivated in pots, were used as test objects. In each case the 3rd pinna was immersed for 30 seconds in a 0·05 % solution of "Systox"

a) The leaves treated in this manner remained on the plants until no more drops formed. Subsequently the content of active ingredient was measured after the liquid ashing, the result being 0·061 5 mg per gramme leaf.

b) After one hour, when the applied solution had dried completely, there was still a radioactivity corresponding to 0·033 2 mg of active ingredient per gramme leaf tissue. Thus 54 % of the quantity of "E 1059" applied had remained on the leaf, the remainder having evaporated. The centre of interest was now focussed on determining the quantity of active ingredient that had penetrated into the leaf after one hour.

The quantity of radioactive marked "E 1059" that had penetrated into the leaf was determined in the following manner. The leaves were thoroughly washed in 10·5 ccs of a 1 % solution of emulsifier using a marten hair pencil; 10 ccs wash water were placed in readiness for the measurement. In the check tests in which the leaves were given a subsequent washing with ether or chloroform, it was only possible to measure traces of radioactivity. The emulsifier solution offers two advantages over organic solvents, firstly one need not fear any decrease in volume due to evaporation during the period of washing and the time of measurement, and secondly any substance that may have already penetrated into the leaf is prevented from being extracted again. Therefore, all subsequent tests were carried out in this manner. After the leaves had been washed in the emulsifier solution, they were throughly rinsed again under running water, and then liquid ashed.

Of the 54 % of active ingredient which remained on the leaf, approximately 75 % (0·024 6 mg out of 0·033 2 mg) penetrated into the leaf. This corresponds to approximately 40 % of the applied solution. Table X shows, as a function of time, the power of penetration of the substance into the leaf and the residue left on the surface. The graph in Fig. 10 also provides a survey of the tabulated values.

Table X. The penetration of radioactive marked "E 1059" into the green leaf of *Vicia faba.*

Time after application	Quantity of active ingredient in wash water		in leaf		total	
	mg/g	%	mg/g	%	mg/g	%
1 hour	0·007 8	23·5	0·025 4	76·5	0·033 2	100
6 hours	0·004 3	13·0	0·020 3	61·2	0·024 6	74·2
24 hours	0·002 4	7·3	0·022 0	66·4	0·024 4	73·7
72 hours	0·002 1	6·35	0·020 6	62·0	0·022 7	68·33

The given results represent average values obtained from three measurements. It must be understood that the values obtained are subject to the margin of error caused by the small number. This applies particularly to the data in Table X, which do not indicate a continuous decrease in active ingredient during the time in the leaf. Within the scope of the complete test programme, it was necessary to forgo a large number of

measurements since in this case at least 30 measurements would have had to be taken for each unit of time. Not until then would the individual differences in the leaves, with respect to their absorbability which depends largely upon the turgescence of the tissue, have been balanced.

It was established in the evaluation of the results that those obtained are sufficient in themselves to account for the penetrativity of "E 1059" into the leaf.

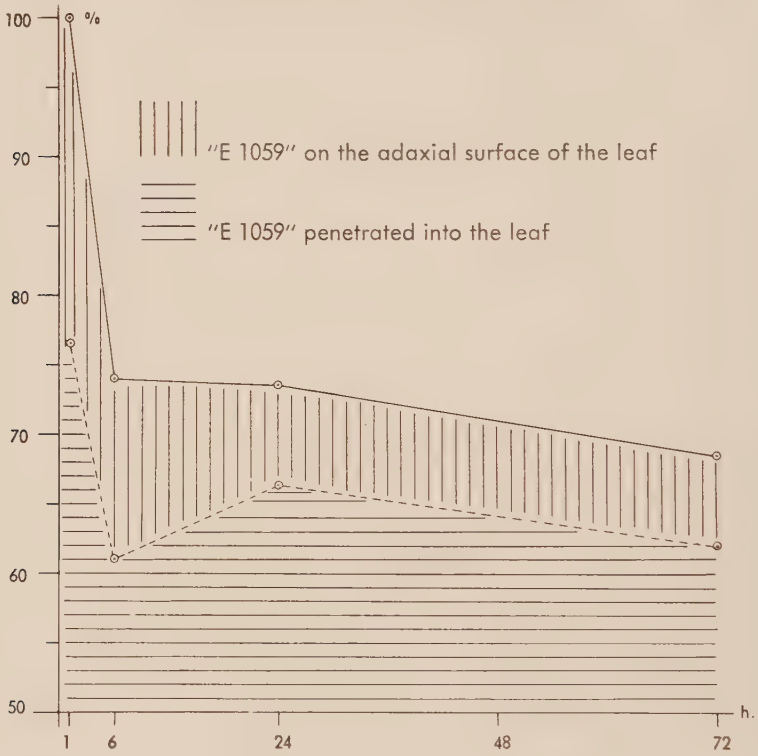


Fig. 10. The distribution of preparation on the leaf. The first measurement was taken one hour after the spray coating had dried on. The measurements were carried out over a period of 72 hours. The original quantity of active ingredient applied is twice as large as that determined in and on the leaf one hour later.

II. Mechanism of penetration

From the preceding test, it had been established that the active ingredient can be traced in the leaf not only in large quantities but also within the shortest possible time. No statement, however, can be made in this respect about the mechanism of penetration. Z a t t l e r (1951) is of the opinion that the stomata should be regarded as the inlets for the active ingredient. He reports that when hop plants are atomized mainly from underneath the leaves, it is possible to attain a longer period of efficacy for the preparation in the plant. The hop leaf has no stomata on its adaxial surface, whereas on the abaxial surface there are large numbers of them. In view of the fact that he had not worked with a radioactive preparation, Z a t t l e r made no direct assertions with respect to the quantities applied but was merely able to establish the long period of efficacy.

Since it is possible to determine the preparation quantitatively by means of the radioactive marked compound, it was decided to analyse more closely the observations of Z a t t l e r. A 0·05 % solution of preparation was applied to the hop leaf. "Systox" solution was coated first on the adaxial surface and then on the abaxial surface. Table XI gives the values obtained from the treated leaves.

Table XI: The penetrative power of active ingredient on the adaxial surface and the abaxial surface of the hop leaf.

	Quantity of active ingredient			
	in wash water		in leaf	
	mg/g	%	mg/g	%
Upper surface of leaf	0·005 8	31·4	0·012 7	68·6
Lower surface of leaf	0·016 1	21·3	0·059 3	78·7

It was, therefore, established in the test that the quantity penetrating the underside of the leaf was only 10 % higher than in the case of the adaxial surface, although as an absolute value the quantity which had penetrated into the leaf was almost four times as much. This effect can be explained, to begin with, by referring to the mechanism involved. Due to its hairy covering, the underside of the leaf has a far greater surface. Furthermore the basal cells of the hairs and the stomata ridges are sites capable of extensive resorption (S t r u g g e r 1939, S t e u b i n g 1949). Added to this, the veins on the underside of the leaf protrude to a large degree whereby very many sharp angles are formed which serve as suitable points of adhesion for the applied quantity of preparation. On the other hand, most of the spray wash runs off the upper surface only leaving a thin deposit behind. Furthermore the adaxial surface of the leaf dries far more rapidly than the abaxial surface which is fairly well protected against the wind. Thus the active ingredient has far more time in which to penetrate into the leaf.

Table XII: Varying absorbability of different leaf types. A 0·1 % solution of radioactive marked "Systox" was applied by the immersion method.

	Family	Species	Quantity of active ingredient in wash water mg/g	in leaf mg/g
Monocotyledons:				
	Commelinaceae	*Rhoeo discolor*	0·005 8	0·003 4
	„	„ „	0·004 2	0·004 1
	„	„ „	0·005 0	0·004 0
	Orchidaceae	*Cypripedium harrisianum*	0·005 9	0·003 5
Dicotyledons:				
	Primulaceae	*Cyclamen persicum*	0·019 6	0·010 1
		„ „	0·009 0	0·013 0
		„ „	0·008 4	0·011 3
		Primula obconica	0·017 0	0·019 8
		„ „	0·012 3	0·038 4
		„ „	0·011 8	0·022 1

III. Dependence of the absorption of "E 1059" upon the leaf type

To compare the absorbability of different leaf types, plants of different families or different genera were examined in October, 1952. In these tests a 0·1 % radioactive marked solution was applied by the immersion method. After 24 hours each leaf was examined for the quantity absorbed. Table XII gives the individual measurements (not average values).

It was clearly shown that the nature of the leaf plays a great part in the property of absorption. The primrose is able to absorb almost three times as much as the cyclamen. The primrose leaf is very hairy whereas the cyclamen has a smooth, leather-like cuticle. Accordingly, the total quantity adhering to the leaf differs greatly. Among the monocotyledons, a leaf type is encountered which is very difficult to wet (cf Linskens 1950; Frohberger, Erna, 1951). The same result was obtained with *Cypripedium* and *Rhoeo discolor*. In this test series, the quantity of active ingredient adhering to the leaves of the monocotyledons was only one-third of that adhering to the dicotyledons, added to which the quantity which penetrated into the leaf was relatively low. It was below 50 % of the dried-on spray coating.

IV. Explanation of the results

The ability of the leaf to absorb active ingredient depends primarily upon two factors, firstly upon the wettability of the leaf concerned, and secondly upon the adhesiveness for the applied substance. These two factors alone, however, are not sufficient to explain the mechanism of absorption or penetration. On considering the studies of David (1951, 1952), who established a much slower absorption of a lower degree through the leaf with respect to "Schradan" and fluorophosphoric acid amide, it is evident that the power of penetration depends upon the lipide-solubility of the substance. The aforementioned compounds are extremely soluble in water, whereas "E 1059" as a thiono compound is only soluble in water in a ratio of 1 : 15,000, and as a thiolic compound in a ratio of 1 : 500. Even when the entire quantity of preparation is in an aqueous solution, the lipophile phase is stronger. This has already been illustrated with respect to the root. The "E 1059" dissolved in water can easily be extracted with organic solvents which are not miscible with water. Since the cuticle, however, contains a large number of lipoids, it should be particularly capable of absorbing the lipophile "E 1059". Thus the question is raised whether the cuticle absorbs the active ingredient uniformly over its whole expanse. Since the discovery by Arens (1929), it is known that phosphatides, for example, are not concentrated homogeneously over the entire leaf surface. Strugger (1939) proved, with the aid of the fluorescence-optical analysis, that dyestuffs are not absorbed uniformly by the cuticle but that within the cuticle there are always sites to be found which must be characterized by an exceptionally large number of pores. Here the penetration of fluorescence dyestuffs can be followed quite clearly. These sites comprise, in particular, the stomata ridges, the basal cells of hairs, and also the anticlines of the epidermis cells. These are the sites where the cuticular transpiration, the recretion of salts and the excretion of organic compounds primarily take place (Strugger 1939; Arens 1934; Frey-Wyssling 1937).

According to what has been said above, the fissures between the guard cells cannot be considered for the absorption of "E 1059" in aqueous emulsion (cf Page 30). The absorbability is only dependent upon the number of stomata

insofar as the stomata ridges represent the primary sites of absorption. Added to this is the fact that the hairs on the abaxial surface of the leaf are instrumental in causing the spray solution to adhere for a long period, and also other peculiarities of the underside of the leaf, e. g. curled leaf edges, protect the active ingredient solution for a long period against evaporation. These characteristics are not to be found on the exposed, and mostly smooth adaxial surface of the leaf. Theoretically, if the adaxial and abaxial surfaces were to offer the same possibility for adhesion, the absorption power of the abaxial surface would be considerably greater anyway due to the large number of stomata ridges and basal cells of the hairs. This is supported by the fact that, in the experiment, four times as much active ingredient was absorbed, although relatively this quantity was only 10 % greater as compared with that absorbed by the adaxial surface of the leaf.

G. The motility of radioactive marked "E 1059" following the treatment of shoot parts

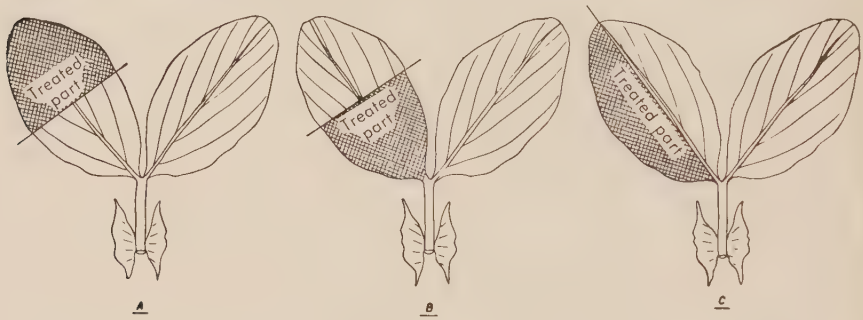
I. Movement in the leaf following application to the upper epidermis

Before an explanation can be given with respect to the mechanism of translocation of the active ingredient in the plant following an application to the leaves or other green parts, an attempt must be made to elucidate the phenomenon of the power of penetration and the possible propagation in the tissue. It has already been mentioned that when the active ingredient is applied to the roots, the leaves of a plant have a far greater concentration of radioactive marked substance to show than all the other organs. This discovery is explicable since the water conducting system of the plant terminates in the leaf, and the high content of active ingredient can be explained quite simply in that a drift occurs. One cannot, however, speak of a circulation of "Systox" in the plant. On the contrary, there are many sites in the leaf where the active ingredient accumulates, at least temporarily.

In order to examine more closely the behaviour of the leaves, a test was carried out whereby the third leaf of 14 day old *Vicia faba* plants was coated in each case on the upper side with 0·5 % solution of radioactive marked "Systox", in the manner illustrated in the figure supporting Table XIII. A strip, 2 to 3 mm wide, of slightly liquefied wax was applied at a temperature of approximately 35 ° C to each leaf, before the treatment, to prevent the applied solution from running. Table XIII, A—C, gives details of the parts treated, and of the results obtained. In the plants treated, according to A, the quantities of active ingredient moving into the untreated basal part of the leaf are hardly worth mentioning. 0·010 mg is the limit at which the preparation can become insecticidally effective. In four other cases there was hardly any spreading. The preparation either did not penetrate into the right-hand leaf pinna at all or if it did it was only when there were large quantities in the basal part of the treated leaf. In section B of the table, the limit of insecticidally effective concentrations in the untreated, upper part of the leaf pinna was reached in all cases. Hardly any active ingredient moved into the other leaf pinna, however. This phenomenon indicates that the preparation is translocated in the leaf chiefly by the transpiration stream. The diffussion property of "E 1059" in the tissue is only slight due to its great lipide-solubility. The values given in section C show that the preparation is

not able to cross the centre vein when applied to only one side, irrespective of whether the leaves are intact on the plant or whether they are isolated. Preliminary tests carried out on leaves of the apple variety "Fey's Frühe" produced the same results as those obtained with the leaves of *Vicia faba* plants.

Table XIII: Movement of the active ingredient in partially treated leaves.



Nature of application	Treated part mg/g	Untreated: left-hand mg/g	leaf pinna %	right-hand mg/g	leaf pinna %
A.	0·282 0	0·003 7	1·3	0	0
	0·192 6	0·020 7	10·8	0·003 6	1·9
	0·175 4	0·001 8	1	0	0
	0·264 7	0·010 2	3·9	0	0
	0·124 1	0	0	0	0
	0·225 5	0·002 4	1	0	0
B.	0·163 7	0·019 2	11·7	0·000 6	0·3
	0·169 9	0·014 1	9·4	0	0
	0·359 6	0·028 0	7·8	0·000 6	0·2
	0·190 8	0·011 2	6·6	0	0
	0·129 8	0·025 4	19·5	0	0
	0·092 3	0·017 0	18·5	0	0
C.	0·180 6	0·011 8	6·6	0	0
	0·169 3	0	0	0	0
	0·249 5	0·004 5	1·8	0	0
	0·230 9	0	0	0	0
	0·307 1	0	0	0	0
	0·215 0	0	0	0	0

The first tests on the movement of the active ingredient in the plant following a treatment of the upper epidermis were carried out without the application of wax borders. In this way it was possible to perceive quite clearly how the preparation slowly spreads over the surface, particularly in the depressions of the nerve system.

The movement of "E 1059" in the leaf cross-section was established by means of autoradiographs (Fig. 11). They showed that the active ingredient penetrated to a full extent through to the underside of the leaf, in other words that it had moved through the leaf cross-section in a matter of 12 hours.

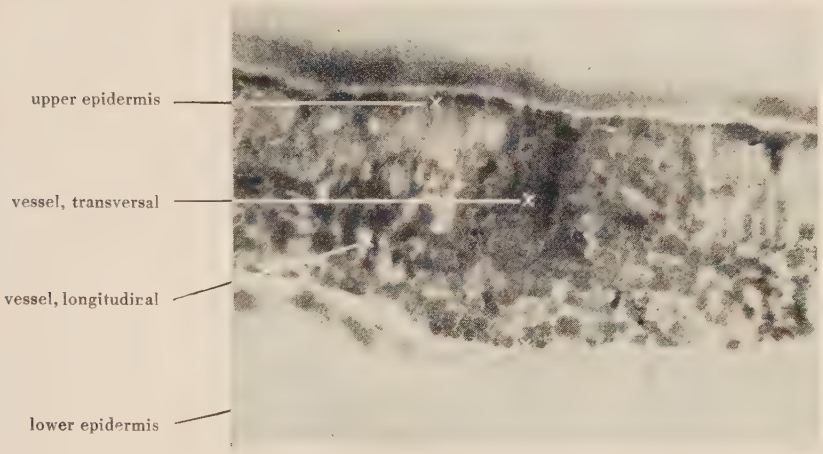


Fig. 11. Autoradiograph of a 10 μ thick leaf cross-section of *Pirus communis*. Magnification × 120. The ^{32}P marked active ingredient was applied 12 hours before the preparation of the upper surface of the leaf by coating.

It was thus shown in this test series that with respect to the diffusion in the leaf, "E 1059" is considerably inhibited. Apparently the active ingredient is arrested temporarily by forces which are to be found in the living cells. The active ingredient is able to move from the basal part of the leaf to the apex through the medium of the transpiration stream.

II. Translocation following treatment of different leaves of *Vicia faba*

The five bottom leaves of 10 day old *Vicia faba* plants were immersed for 30 seconds in a 0·05 % solution of radioactive marked "Systox'. The whole of the shoot axis, a fully developed leaf, a half developed leaf and the shoot apex were left untreated. The two leaves were located immediately below the shoot apex (see Fig. 7). The translocated quantities were measured after 3 days. The first test series showed the following:

0·157 6 mg active ingredient had penetrated into 5 g leaf. 0·040 mg accrued in the wash water. 0·037 0 mg active ingredient was traced in the untreated parts — shoot axis, 6th and 7th leaves and shoot apex — which weighed 8·5 g. This quantity of 0·037 0 mg represents the average of 3 tests. Therefore, 15·6 % of the whole quantity of active ingredient found in the plant was translocated.

The maximum values of translocated active ingredient in the untreated leaf, with an applied concentration of 0·05 %, are between 0·010 and 0·012 7 mg/g. This means that when a normal spray treatment is carried out, whereby the shoot axis is also included and no large parts of the plant are left untreated which is the case here, there should be an insecticidally effective concentration

of active ingredient in all parts of the shoot. This applies in particular to new organs developing at the shoot apex, which can be supplied with an adequate quantity of active ingredient from the older parts.

In contrast to the preceding test, only about half of the leaves were treated in the following test. They were immersed as before for 30 seconds in a 0·05 % solution of radioactive marked "Systox". The movement of active ingredient up the shoot proved, in this case, to be considerably extensive. It amounted to about one-third of the total quantity of active ingredient present in the plant. It was not sufficient, however, to render the untreated parts insecticidally effective.

The next question as to whether the movement of active ingredient towards the basal part was just as extensive was clarified in the same manner with *Vicia faba* plants. The apex and the two upper leaves were dipped for 30 seconds in the 0·05 % solution of radioactive marked "Systox". The plants were laid out horizontally until the solution had dried on. Only 16·2 % of displaced "E 1059" was traced. Large quantities of active ingredient were only found in the leaf located immediately below the treated ones.

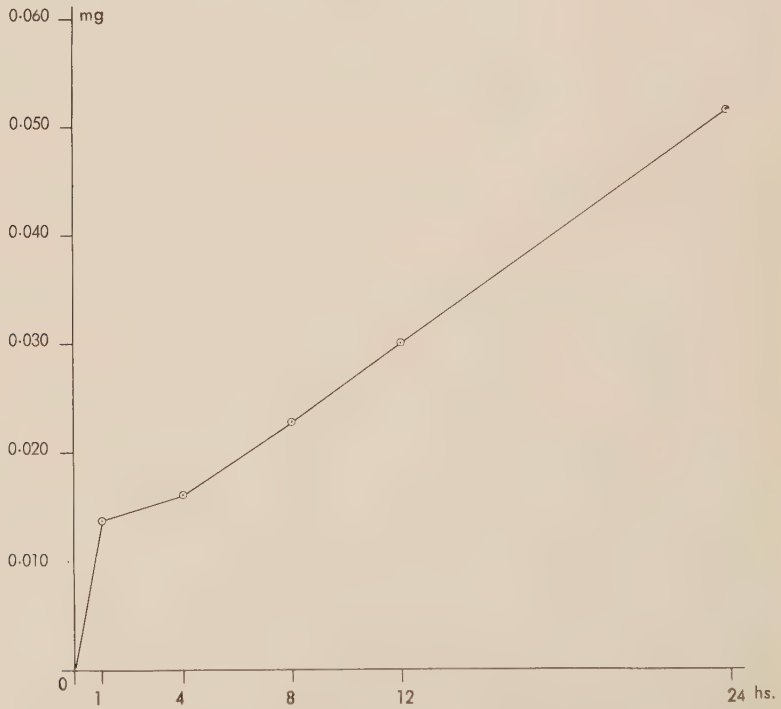


Fig. 12. Translocated quantities of preparation in the untreated parts of *Vicia faba* plants following an application of 0·800 mg active ingredient to the three bottom leaves.

The speed at which the preparation is translocated is shown in Fig. 12. The plants were examined 1, 2, 4, 8, 12 and 24 hours after the application of 0·800 mg active ingredient.

The findings only apply to *Vicia faba* plants, but to test the behaviour of other plants experiments were carried out on *Phaseolus vulgaris* and *Tropaeolum majus*. A few specimens of *Phaseolus vulgaris*, which had just formed their primary leaves, were immersed with one primary leaf for 30 seconds in a 0·5 % solution of "Systox". 24 hours later, the measurement was carried out on the other leaf. Whereas 0·120 mg active ingredient per gramme leaf was determined in the treated leaf, only 0·001 2 mg/g leaf could be traced in the untreated one. In the case of *Tropaeolum majus*, treated in a similar manner, 0·110 mg/g of active ingredient was present in the treated leaf, whereas only 0·001 5 mg/g could be traced in the untreated leaves lying above it. These tests showed, therefore, that also for *Phaseolus* and *Tropaeolum* the movement of "E 1059" is very slight following partial treatment.

III. Conducting elements in the plant

It could be established in Section G 1 on the basis of the translocation capacity of the active ingredient that an affinity to the cell content is probable. The relatively slight degree of movement does not result from diffusion but is effected by special conducting elements in the plant, and these can only be the xylem and the phloem. Translocation from the leaf through the xylem can only occur when there is an inverse transpiration stream in the leaf itself. Such cases have only been established where there is a water deficit in other parts of the plant, chiefly in the region of the roots (cf Strugger 1939). The translocation of radioactive substances from the leaf takes place in the phloem, a process investigated by Biddulph and Markle (1944), and by Chen (1951). Here motile sugar, amino acids and phosphate salts are supplied chiefly to newly formed organs in growing plants. When the substance is translocated from the leaf stem and flows into the shoot axis, a part of it is able to pass into the xylem, and from here a rapid process of translocation begins in the transpiration stream. The greater part of the substance, however, remains in the phloem where it moves in an upward and a downward direction.

It was by no means expected that "E 1059", a substance completely extraneous to the plant, would react in a conformable manner. In order to study the movement from the leaf surface to the petiole and in the shoot axis, tests were arranged with willow sets. Radioactive marked "E 1059" was applied to plants on which the bark was separated in places from the wood. Three longitudinal sections were made, the bark was separated from the wood by means of a spatula, and a piece of paraffin-soaked parchment paper was inserted between the wood and the bast. Then the processed site was bound with cellophane paper. Moist cellulose packing was placed around the outside, which had to be re-moistened from time to time. The processing was carried out 12 hours before the test.

A twig, growing either above or below the operated internodium, of each set was immersed for 1 minute in a 0·2 % solution of radioactive marked "Systox". The examination was carried out 30 hours later, whereby the leaves of the treated twigs, the leaves of the untreated twigs, as well as the bark and the woody part on the processed sites were checked separately for their content of active ingredient. In figures 13 A and B the relevant parts of the plants are designated by Roman numerals. The values obtained are reproduced in Table XIV, A and B.

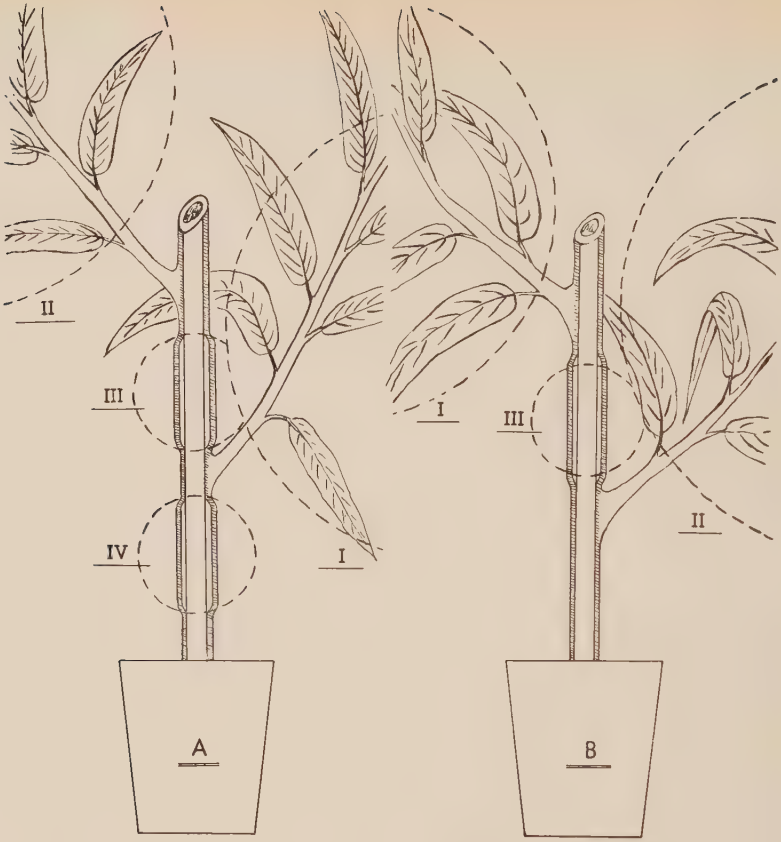


Fig. 13. The explanation to the values in Table XIV is provided by the willow sets, marked A and B
I. Treated twig.
II. Untreated twig.
III. and IV. The bark is preparated from the wood by paraffin-soaked parchment paper.

Table XIV: Translocation of "E 1059" in willow sets *(Salix viminalis)* with loosened bark.
(Explanation in Fig. 13)

A.	I treated leaf mg/g	untreated II leaf mg/g	III bark mg/g	III wood mg/g	IV bark mg/g	IV wood mg/g
1st plant	0·078 0	0·000 40	0·000 25	trace	trace	0·000 25
2nd plant	0·085 0	0·000 75	0·000 25	0	trace	0
3rd plant	0·080 0	0·001 20	trace	0	0·000 25	0

B.	treated leaf mg/g	untreated II leaf mg/g	untreated bark mg/g	untreated III wood mg/g
1st plant	0·075 3	0·000 42	0·000 56	0·000 25
2nd plant	0·078 6	0·001 73	0·000 30	0·000 37

As a result of the processing carried out on the plant, it is only natural that conditions can be created in the plant which, with respect to productivity, must be regarded as abnormal. This applies in particular to the separated phloem. Provided allowances are made for this, it may be said — this assertion being based upon the experiment described above — that the radioactive marked active ingredient is present both in the phloem and in the xylem below and above the treated twig. On the whole, the phloem, or the bark, may be said to contain more active ingredient than the xylem. It should be borne in mind, however, that the reference units (gramme of substance) for the xylem and the phloem are most comparable. In a further experiment, an attempt was made to establish more exactly how the phloem and the xylem of the shoot axis behave.

The test plant used for this purpose was *Pelargonium zonale*. A strip of bark, 1 cm wide, was removed from the circumference of 4 month old sets. The shoot axis was bound to a width of 4 cm with cellophane paper and a moist cellulose packing, this being done in such a manner that the paper did not touch the wound. Except for the shoot apex and a small leaf which was in the process of forming, all the leaves located above the ring were removed (see Fig. 14). Two leaves below the ring were immersed for 30 seconds in a 0·5 % solution of radioactive marked "Systox". Although the bark of the check plants was not touched, the plants themselves were treated in the same manner as described above, i. e. all the leaves located above the two treated ones were removed. 24 hours later, the plants were examined for the translocated quantity of active ingredient in the shoot apex. The following values were obtained:

Table XV. Translocation in *Pelargonium zonale* (Explanation in Fig. 14).

Plants	Leaf, treated mg/g	Shoot apex, untreated a) non-ringed mg/g	b) ringed mg/g
1		0·003 8	
2		0·002 8	
3		0·003 5	
	M = 0·080		
4			0·001 4
5			0.001 1
6			0·000 6

It can be clearly seen that the movement towards the apex starts primarily in the phloem. As an average value, one arrives at a ratio of wood movement to bark movement amounting to 1 : 2·2, i. e. twice as much is transported through the phloem as through the xylem.

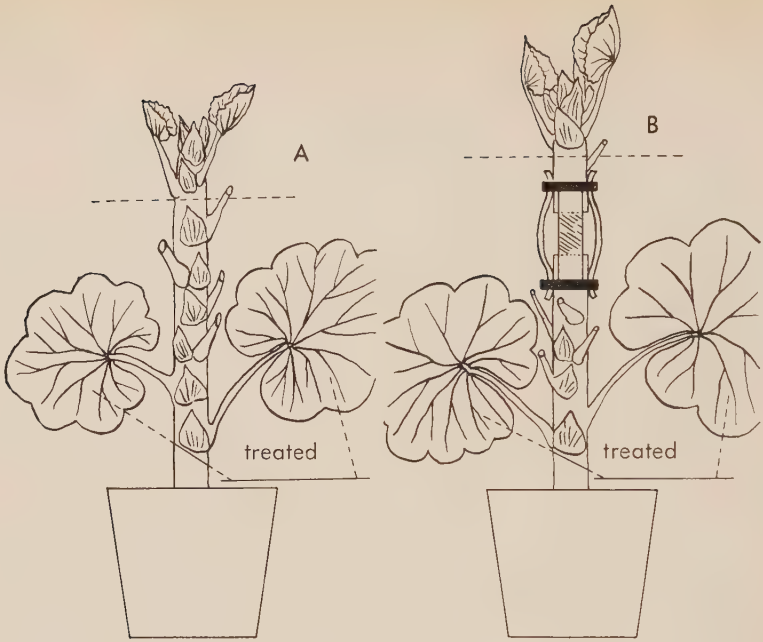


Fig. 14. Explanation to Table XV.
That part of the plant marked A was examined for its content of preparation. The stem was intact, and merely the untreated leaves were removed.
Part B was examined in the same manner. A 1 cm strip of bark has been removed from the stem, however.

IV. Discussion of the results

The tests have shown that the active ingredient is translocated in the plant up to a certain percentage. The more extensive the treatment of the leaves, the greater was the translocation. When the leaves were only partially treated with active ingredient, the translocation was insignificant. This may be explained chiefly by the behaviour of certain structural elements in the leaf, namely the connecting parenchyma of the vascular bundle. They represent the decisive threshold for the movement.

Zech (1952) also arrived at findings of a similar nature. He studied the spreading of tobacco mosaic virus in the primary infected leaf. After reaching the nerves, the tobacco mosaic virus continued to move in the direction of the connecting bundle parenchyma only, and was chiefly translocated there. It moves quickest in a basal direction. Generally, there was no propagation vertical to the nerves, in other words across them, and when this did occur it was only after some considerable time.

The autoradiographs produced with "E 1059", e. g. Fig. 11, show that following an application of active ingredient to the adaxial surface of the leaf there is an increased storage of active ingredient in the vascular region.

The studies of Wanner (1952), Biddulph and Markle (1944), and Chen (1951) give a clear picture of the way "E 1059" moves in the leaf. Wanner found that the phosphatase activity in the connecting bundle parenchyma increased greatly. The phosphate sugars which pass into the connecting bundle parenchyma from the sites of assimilation as a result of diffusion are

cleaved there. The sugars reach the phloem stream in the form of disaccharide. The fact that the radioactive phosphoric acid which Biddulph and Markle, and also Chen, applied through the leaves can be traced in the sap of the sieve tubes and finally also in the water of the xylem is interpreted by Wanner in the following manner. The phosphoric acid separated from the phosphatase is flushed passively with the sugars into the phloem, and during the crossover of water to the xylem it also reaches the woody part. In a similar manner, we can also imagine how the active ingredient in the epidermis cells moves from cell to cell as the result of diffusion. In the connecting bundle parenchyma, which has a special absorption and storage property for "E 1059", it is flushed passively into the phloem stream and from there on a small scale into the transpiration stream. The connecting bundle parenchyma is passed when the quantity of "E 1059" present in the leaf is so great that the parenchyma is not able to store any more. The particular affinity of the cells of the connecting bundle parenchyma to "E 1059" remains a mystery.

H. The long lasting effect of "E 1059" in the plant

I. Storage of radioactive marked "E 1059"

The preceding tests have proved that the roots and the leaves are able to absorb the active ingredient in large quantities. The active ingredient is translocated in the plant throught the xylem following an application in the region of the roots, whereas following a leaf treatment it is transported chiefly in the phloem. It now remains to answer the question how long cells which have either absorbed the "E 1059" directly or from the conducting elements retain the toxic substance in presumedly active form, or when they are to be regarded as detoxified.

Potted plants in greenhouse cultures which have been wetted with 100 cm^3 0·1 % solution of "Systox" are not infested by red mites and aphids for 3 to 4 weeks. It is not to be expected that a depot of active ingredient can remain in the soil for a long period when it is watered daily. On the contrary, it would be greatly diluted, and any quantities of active ingredient absorbed by the roots would not be sufficiently concentrated to become insecticidally effective. The plant has no actual storage organs in which foreign substances can be accumulated only to be mobilised and translocated again at some time or other. It was to be assumed that the plasma which contains an abundance of lipoids would be able to absorb "E 1059" and store it temporarily. Autoradiographs of microtome cross-sections through the pear leaf (Figs. 15 to 17) were used to prove how far this assumption is correct. They show that the living cells of the leaf, particularly those around the vascular bundle, store the active ingredient.

This otherwise excellent method of bioassay unfortunately has two great disadvantages. Firstly the substance being traced must posses a high specific radioactivity, On the average it is 1 μ C/g tissue. The activity can only be attained with radioactive marked "Systox" — where, with a molecular weight of 258, only the phosphorus (M = 32) possesses the radiation — by overdosing the substance, in other words exceeding the phytotoxic limit. Naturally the fixation of the tissue is carried out before any visible injuries appear. Allowance must be made, however, for damage to the plasma. Secondly, artificial radioactive phosphorus requires a long exposure time, on the average 14 days. Any postmortal processes occurring during this period cannot be controlled. Changes of a post-mortal character can also occur during the fixation itself. Under certain circumstances, the plasma can allow large quantities of substance to diffuse in the vacuole while the fixation is being carried out. Such phenomena must be allowed for with radioactive marked "Systox".

With the aid of the stripping film in the autoradiograph, it was possible to explain, at least generally, the storage of "E 1059".

The radioactive marked active ingredient does not emerge from the vessels by first moving to the ends of the conducting bundles but passes out of the vessels into the surrounding parenchymatous cells on a more or less large scale throughout. The degree of translocation, however, is greater in the region of the leaf periphery than in the interior of the leaf. We found that in every case the regions around the leaf periphery were blackened to a greater extent than the parenchymatous cells in the vicinity of the centre nerve. The path taken by the active ingredient to pass from cell to cell is undoubtedly through the intercellular spaces of the cell walls. Due to the lipidesolubility of "E 1059", a portion is extracted from these submicroscopic paths by the plasma where it is stored. After the plasma has become saturated, the substance is also passed into the vacuoles (Figs. 15, 16, 17). In all the photographs of the autoradiograph it was seen that very large concentrations of active ingredient are contained in the cells of the connecting bundle parenchyma. It is improbable that this phenomenon is solely due to the affinity of the active ingredient to the lipophile phase. There must be other forces present which are peculiar to the cells of the connecting bundle parenchyma exclusively. The same can be said to apply to the epidermal cells (Figs. 15, 16, 17).

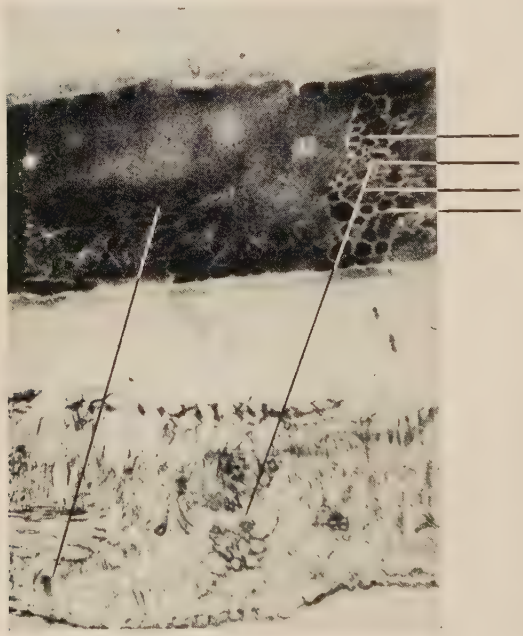


Fig. 15.

Autoradiograph of a leaf cross-section of *Pirus communis*. (The blackened stripping film is shown at the top with the corresponding section below it. The section has been damaged due to the film being raised. One vessel is cut longitudinally and another transversally. The lines join the longitudinally and transversally cut vessel from the object to the film.) Here the large accumulation of preparation in the connecting bundle parenchyma is most noticeable. The application was made by placing a twig in a ^{32}P marked "Systox" solution for 12 hours. Thickness of section 10 μ, magnification × 120, time of exposure 12 days.

It is assumed that the storage of "E 1059" is determined on the one hand by its lipide-solubility, and on the other hand by its affinity to substances with an active surface. The following experiment illustrates how the active ingredient is linked in different degrees to three substances with active surface.

50 ccs of a 0·0001 % solution of radioactive marked "Systox", poured into each of three porcelain suction filters 10 cm in diameter which beforehand had been lined with hardened filter paper and in which 10 g of starch, active carbon or diotomaceous earth had been elutriated, contained far less active ingredient in filtrate. Thus 21·2 % of "E 1059" is retained by starch, 53·5 % by carbon and 76·4 % by diatomaceous earth.

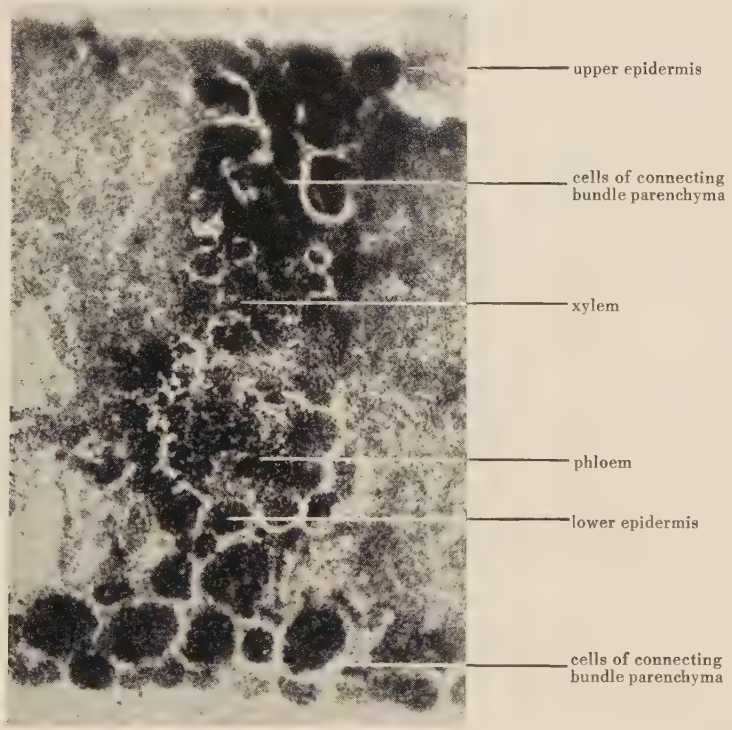


Fig. 16. Section magnification of a transversally cut vessel for a leaf of *Pirus communis*. The same object as in Fig. 15, except that here the stripping film has remained on the section. (Section thickness 10 μ, magnification × 540, time of exposure 12 days.)

II. Detoxication of the plant

When a part of a shoot wetted with "Systox" solution is cut off, and placed in a glass vessel closed at the top with gauze material with a second vessel containing house flies on top of it, the fies die within a relatively short time. This effect can only be explained by assuming that the active ingredient is discharged in the form of vapour. It remained to be found out what part is played in this by the transpiration processes or the sites of transpiration.

An attempt was first made to determine the discharged active ingredient quantitatively:

The experimental apparatus set up for this purpose is illustrated in Fig. 18. A constant current of air was produced with the aid of compressed air, its intensity being checked by means of a Riesenfeld flowmeter. Whenever dry air was required a drying tower containing $CaCl_2$ had to be connected up. A glass bell with a face ground periphery, and containing the test object, was sealed against a glass plate with Ramsay grease. In the top of the glass bell was an opening through which a tube led to a washing bottle (height 30 cm). The washing bottle was filled with glass beads, 0·5 cm in diameter, to make the surface in the bottle as large as possible. The washing bottle was immersed in a Dewar vessel containing a cold mixture (dry ice-acetone of -80^0 C. The tube continued via a pressure-equalizing reservoir to which a water jet pump was connected. The pressure-equalizing reservoir and the water jet pump prevented excess pressure occurring under the bell. It should be mentioned that very few rubber connections were used in the tubing between the glass bell and the washing bottle since rubber tends to absorb "E 1059".

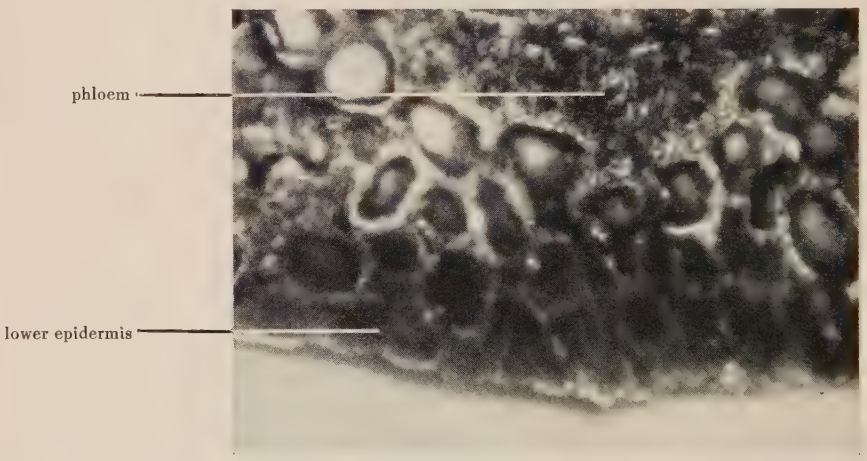


Fig. 17. Autoradiograph of a leaf cross-section of *Pirus communis*. The stripping film is lying on the object. (10 μ; magn. × 540; time of exposure 18 days.)

For reason of its large leaf surface, *Coleus* was selected as the test object. The plants were removed from the pots, the roots were washed as well as possible, and placed for 2 hours in a 0·1 % solution of non-radioactive "Systox". The vessels containing the "Systox" solution and the plant roots were then sealed tight in paraffin, placed under the bell jar, and the apparatus was put into operation. In order to establish whether insecticidally effective quantities could be collected, the apparatus was operated at first in a simplified manner. Flies in a 100 cc Erlenmayer flask behind the bell jar were exposed to the air current. 100 % eradication was obtained in 210 minutes. When the whole apparatus was operated — the temperature under the bell glass at 24^0 C and the relative humidity at 65 % — the yield of condensate was 94 % (determination of weight). 3·5 ccs of liquid were yielded from the washing bottle. Mosquito larvae died in this condensate after 18 hours.

The method described above did not provide any useful information about the quantities discharged. Therefore, the experiments were repeated with radioactive marked "Systox" solution. One plant was left in the 0·1 % solution during the entire period of the test. After an imbibition lasting two hours,

the other plants were transferred to a normal nutrient salt solution to prevent a permanent supply of active ingredient between the root, xylem and stomata. Apart from determining the quantity of active ingredient exudated, another purpose of these experiments was to establish whether a part is played by the condition of the stomata, namely when they are open and closed. Consequently the 2nd test (Table XVI) was carried out with an illumination of 10,000 lux and at a relative humidity of approximately 70 %, whereas the third test took place at night in complete darkness at a relative humidity of 100 %. The stomata were wide open on the illuminated plants, and fully closed in darkness (infiltration method according to Molisch 1930).

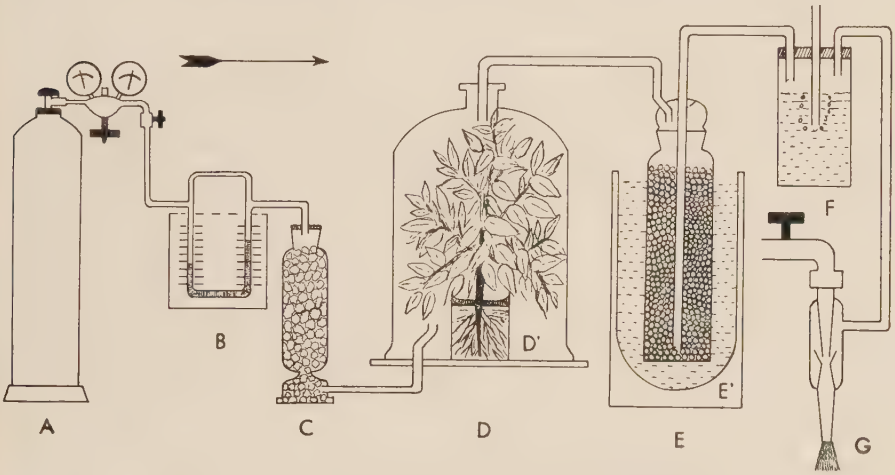


Fig. 18. Test apparatus to determine the preparation exudiated from the plant.

A. Air bottle
B. Riesenfeld's flowmeter
C. Drying tower filled with $CaCl_2$
D. Vacuum-tight glass bell
D'. Plant in a nutrient solution (vessel is sealed with paraffin)
E. Washing bottle, filled with glass beads to condense the "E 1059" vapours
E'. Dewar vessel for cooling to -80^0 C
F. Pressure-equalizing reservoir
G. Water jet pump

The data given in Table XVI prove that the condition of the stomata, whether open or closed, makes no difference to the quantities of active ingredient exudated. In the case of supersaturation, or preferably expressed, when the plant constantly absorbs active ingredient through the root, the rate of exudation is increased considerably (Table XVI, 1 compared with 2).

Although the open or closed condition of the stomata exerts no large influence upon the exudation of active ingredient, there is, however, the possibility that some significance is attached to the number of stomata present, or to the leaf type. Consequently, several leaf types were examined more closely (results given in Table XVII).

Shoots of several plant species were cut off under water, and placed for 24 hours in a 0·1 % solution of radioactive marked "Systox". The upper and lower surfaces of the leaves were then washed thoroughly in 10·5 ccs of a 1 % emulsifier solution, using a marten hair pencil. The activity was determined in 10 ccs of the wash water. The concentration present in the leaves was also checked.

Leaves which have no stomata on their adaxial surface exudate the active ingredient — such as is shown here with respect to the apple leaf — to a far

greater degree through the abaxial surface than through the upper surface. *Vicia faba* and *Phaseolus vulgaris* reveal differences of much the same size on the upper and lower surfaces of the leaf. Herbaceous plants have fewer stomata on the adaxial surface of the leaf than on the abaxial surface. Whereas the proportion of recretion for the apple leaf — which has no stomata on the upper surface — is 1 : 5, for the mentioned herbaceous plants it is 1 : 3·7.

Table XVI: Condensation of radioactive marked preparation exudated by the plant into the air.

Plants	Conc. of sol.	Period of immersion	Temp.	Rel. Hum. %	Period of test in hrs. and intensity of illumination	Yield (Determination of weight %)	Quantity obtained following acetone washing of beads mg	Surface of plant dm^2	Content of active ingredient in 1 g leaf mg
1	0·1 %	2 hrs. before and during the test	22 °C ± 1 °C	60	2·5 10,000 lux	93	0·002 8	15·170	0·034 0
2	0·1 %	2 hrs. before then in nutrient solution	23 °C ± 1 °C	100	6 hrs. at night, in complete darkness	103	0·001 7	16·100	0·005 6
3	0·1 %	2 hrs. before then in nutrient solution	22 °C ± 1 °C	70	6 hrs. 10,000 lux	95	0·001 8	18·006	0·005 7

Table XVII: Exudation of preparation on adaxial and abaxial surfaces of leaf as a function of the number of stomata and of the cutin layer of the epidermis.

Species	No. of stomata per mm^2 Abaxial	Adaxial	No. of leaves	Leaf weight g	Leaf area cm^2	pH of wash water	Quantity of active ingredient Adaxial surface mg	Abaxial surface mg	Leaf content in mg/g	Ratio. of adax surface to abax. surface
Apple	0	250	5	9·4	324·3	7·5—7·6	0·002 27	0·011 5	0·800	1 : 5
Vicia faba	50	70	7	2·9	128·5	7·5	0·004 5	0·016 5	0·275	1 : 3·7
Phaseolus	82	107	4	5	363·5	7·6	0·003 5	0·013 0	0·450	1 : 3·7
Mahonie	0	230	6th pinna = 37 leaves	10	560·9	7·75	0·002 53	0·001 83	0·510	1 : 0·72

We have learned, therefore, that the exudation of active ingredient does not depend upon whether the stomata are open or closed, although the number of stomata present does have an influence upon the extent to which recretion occurs. The active ingredient, therefore, is exudated with the transpiration water through the outer cell walls, and this exudation assumes particularly large proportions at the stomata ridges. The autoradiographs too reveal a systematic accumulation in the parenchymatic cells which are located in the vicinity of the epidermises (Fig. 17).

Once it had been established in what quantities the active ingredient is eliminated in vapour form during the process of transpiration, studies had to be made in order to establish whether there is any temporal connection between exudation and detoxication. For this purpose three test series were carried out. All the plants involved, which again were 10 days old *Vicia faba* were immersed in a 0·05 % solution of radioactive marked "Systox". The first measurement was taken after the solution had dried on, which was approximately 2 hours after the application. The other values were ascertained at intervals of 3, 6, 12 and 20 days. In the first test series, the plants were kept under normal conditions in the greenhouse. The plants of the second test series were exposed to circulated air — produced by a fan — for 8 hours every day. 2000 mm of water were applied daily for a period of 12 days (8 hours per day) to the plant of the third test series. Such abnormal conditions as 2000 mm of rainfall in a day never occur in nature. It was, however, important to show in this model test which meteorological factors play a part in the recretion of the active ingredient from the plant. Fig. 19 illustrates the decrease in active ingredient under the three conditions. Curve D represents a comparison with plants which were exposed to the weather outdoors. Under normal conditions prevailing in the greenhouse (curve A), the exudation is more or less linear until the 12th day, and from then on, once a concentration of 0·010 mg/g has been attained, it proceeds much more slowly. Curve B shows the values for plants subjected to a current of air which outdoors would not be so persistent but just the same cannot be regarded as extreme. Here again there is a sharp drop until a residual concentration of 0·012 mg/g is reached, after 3 days. The remaining decrease in concentration runs more or less parallel to that of curve A until the 12th day. The conditions shown in curve C are quite different since there was an abnormal application of water. Here one can really say that the active ingredient was thoroughly washed out of the plant.

The determined concentrations of active ingredient permit a statement to be made with respect to the time taken before the plants were rendered non-toxic to insects with a piercing-sucking mouth apparatus. In our tests the corresponding concentration was 0·008 mg per gramme plant. Under natural meteorological influences, it may thus be expected that the insecticidal effect will have died off after approximately 7 to 21 days dependent upon the species of plant and insect involved.

III. Discussion and possible significance of the findings

On reading through the descriptions of the tests in the last chapter it is perceived that the greatest quantity of the applied active ingredient was recreted by the green parts at the same time as the plant ceased to be insecticidally effective. However, when the toxicity of the substance is maintained for a longer period in the plant, in fact as long as sufficient quantities of radioactive marked active ingredient can still be traced, it can be concluded that the molecule is stable. Whether the residual quantities still present in the plant, which are also constantly exudated, represent the originally applied molecule, is a point which cannot be decided without a microchemical analysis.

The mechanism of exudation is really a most complicated form of nature, particularly in view of the fact that an influence is exerted by several factors. It would be advisable, first of all, to consider an exudation of substance,

i. e. recretion or excretion. In doing so, the transpiration appertaining to the cuticles and the stomata must also be discussed. As yet no one has been successful in definitely proving whether the recretion or excretion of cations through the intact cuticle is a passive or an active process (Arens 1934, Lausberg 1935, Frey-Wyssling 1937). It is only known that recretion, and also the cuticular transpiration, increases (Härtel 1947, 1951) with the swelling of the cuticles. This is a consequence of the increase in the size of the pores caused by the swelling process. According to this, the cuticular transpiration must be a part of the salt excretion process.

In order to fully understand the exudation of "E 1059", it is above all essential to learn of the observations made by Arens (1929, 1934), who reported that the heaviest emission of salt occurs when the leaf is wetted by dew, fog and rain, in other words also when there is no transpiration. Arens also pointed out that phosphatides are excretory substances which pass through the intact cuticle. He presumed that the anticlines of the epidermis cells and the cells of the stomata are the sites of exudation. The observations of Arens will be of value in answering our question when we also consider the discoveries of Strugger (1939) with respect to the extrafoscicular transpiration stream. He proved with the aid of a fluorescence-optical analysis that considerably large

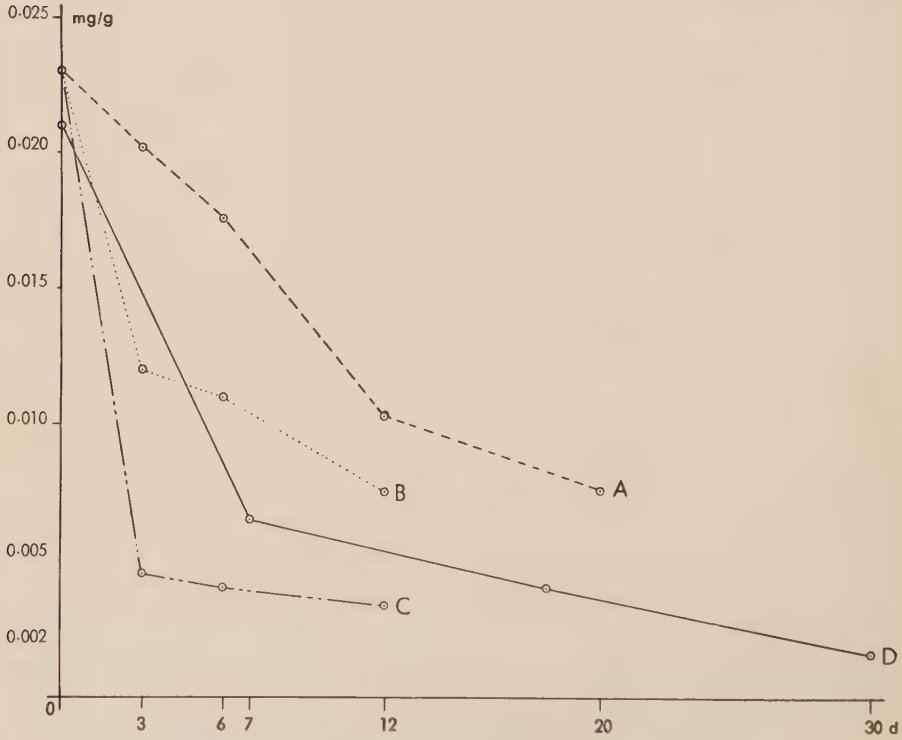


Fig. 19. Excretion of ^{32}P marked preparation from the plant as a function of the time unit and the influence of several environmental factors. Object: *Vicia faba*.
A. in static air in the greenhouse
B. in circulating air in the greenhouse
C. with artificial rain in the greenhouse
D. outdoors

molecules move in the submicroscopic spaces of the membranes, and that they emerge freely at the cuticles. The anticlines of the epidermis cells, the basal cells of the hairs, and the stomata grooves are the sites of exudation.

In comparison to the cuticular exudation, transpiration through the stomata merely plays a secondary role. The plants only discharge "E 1059" dissolved in water when they absorb preparation through the roots during the test. Our tests have proved that the cuticular transpiration does not depend upon whether the stomata are open or closed, since the recretion lies in the same order of magnitude irrespective of whether the transpiration does not cease at all or whether the maximum proportion is obtained with the stomata open.

On summarising the exudation of "E 1059" we obtain the following picture. The active ingredient passes from the plasma into the submicroscopic paths of the cell walls. It cannot be said how it comes to exudate in the membrane stream. It is possible, however, that a linkage to the phosphatides is partly responsible. The membrane stream feeds the "E 1059" to the surface where it is exudated either by transpiration or by washing out. We are not able to prove whether the drop in concentration in the region of the surface is responsible for the exudation of the active ingredient molecule or whether it is due to active exudation forces (exosmosis). It is striking that the extent to which the phosphatides and "E 1059" are recreted is the same for both the adaxial and the abaxial leaf surfaces, irrespective of whether the leaves are slightly or strongly cutinized. This fact can be interpreted by assuming that the phosphatides and "E 1059" are translocated in a manner common to both.

Summary

1. "E 1059", as the active ingredient of the systemic insecticide "Systox" is marked by the incorporation of radioactive phosphorus. Its behaviour is followed when it is applied to the roots or leaves for absorption.

   By using a liquid countertube in the Geiger-Müller counter apparatus, it was possible to detect quantities which were well below the value accepted for insecticidal efficacy.

   Whereas a concentration of 5×10^{-6} preparation solution is sufficient to kill aphids, dilutions of 1×10^{-9} can be traced physically.

2. "E 1059" is absorbed freely from a solution by the intact root. The absorption from sand and humus-containing soils is a slower process, and is effected only up to a certain limited concentration which differs for each type of soil.

   When the active ingredient is absorbed from a soil, the permeability of the root for water increases over a long period, only to drop later below the normal value. It then adjusts itself again. Certain quantities of "E 1509" are recreted by the root.

3. After the active ingredient has been absorbed by the root, it is translocated through the medium of the transpiration stream to shoot organs growing above ground.

4. The leaves are able to store the active ingredient temporarily.

5. "E 1059" is accumulated to a greater extent in the regions of the leaf periphery than in the leaf centre. This is particularly due to the damming of the transpiration steam at the periphery.

6. When "Systox" solution is applied to the leaves by means of a spray or with a brush, the active ingredient is able to penetrate into the interior of the leaf at a higher concentration. In fact 35 to 40 % of the applied quantity penetrates into the leaf within the short space of one hour. A quantity of active ingredient adhering to the leaf or penetrating into the leaf is dependent upon several factors, inter alia, the wettability of the solution, the speed at which the spray coating dries on the leaf, and above all the anatomical structure of the leaf.

7. In the leaf, the active ingredient does not spread over large distances as a result of diffusion, although the leaf cross-section is traversed rather rapidly by the active ingredient. The nerves greatly inhibit further spreading, and with respect to this it may be said that the connecting bundle parenchyma restricts the movement of the active ingredient.

8. When single leaves are treated, the active ingredient moves to the untreated parts of the plant, this movement being more extensive in the apical direction than in the basal direction. The translocated quantities of active ingredient are only insecticidally effective, however, when the greater part of the plant is treated with a solution of a normal concentration.

9. The phloem is primarily responsible for the translocation of the active ingredient following a treatment of the shoot parts growing above ground, although certain quantities also pass into the xylem. It is probable that "E 1059" is translocated passively with the assimilates.

10. "E 1059" is stored temporarily by the living cells. Autoradiographs of leaf cross-sections show that there are particularly large accumulations in the epidermises and in the cells of the connecting bundle parenchyma.

11. The detoxication of the plant takes place chiefly as the result of recretion. In normal cases this must be regarded as purely cuticular. The active ingredient molecules are caught in the extravascular membrane stream and exudated through the pores of the cuticle. A part is probably played by the phosphatides in the elimination of the active ingredient. The active ingredient is removed from the surface either by rain or as a result of evaporation.

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